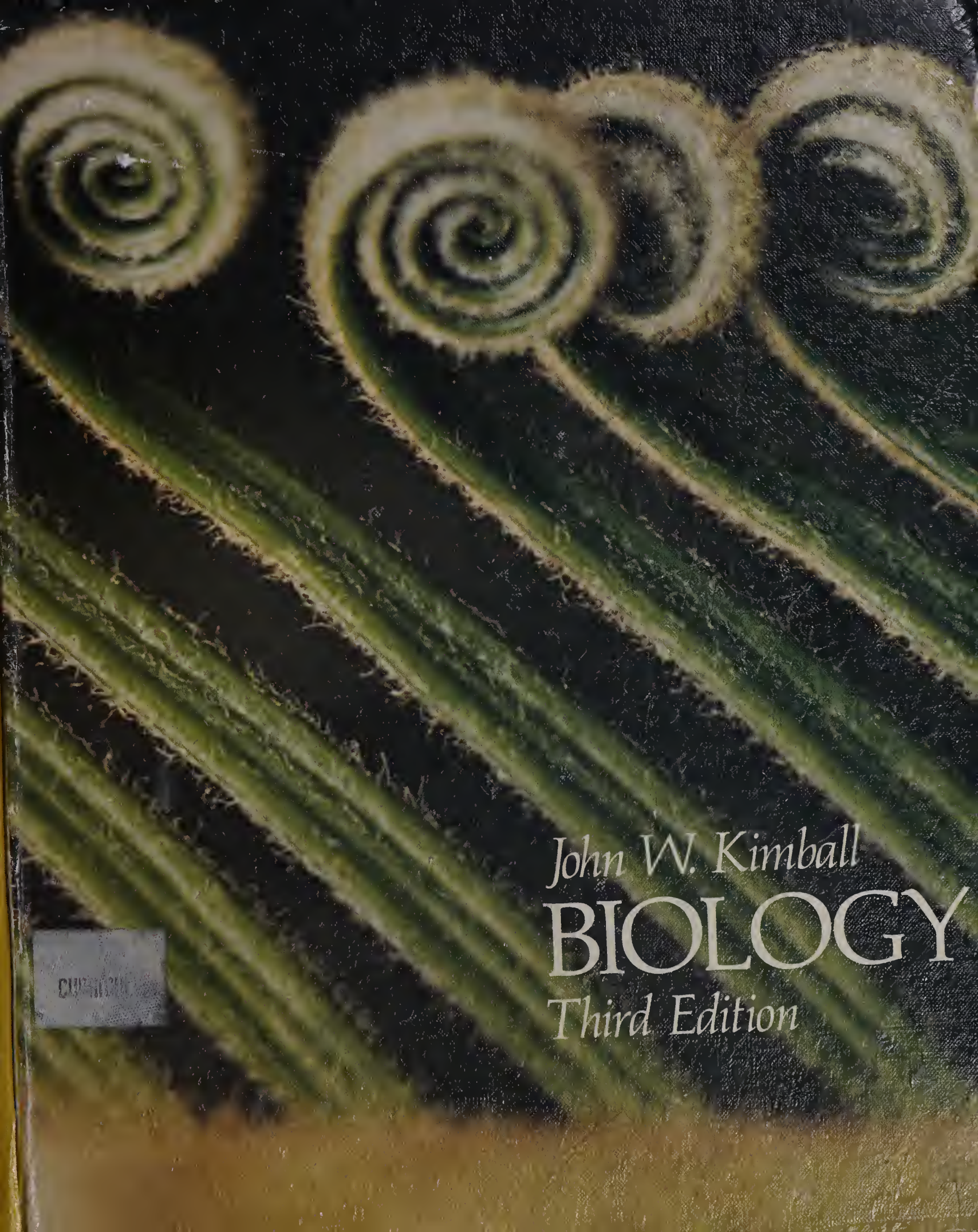
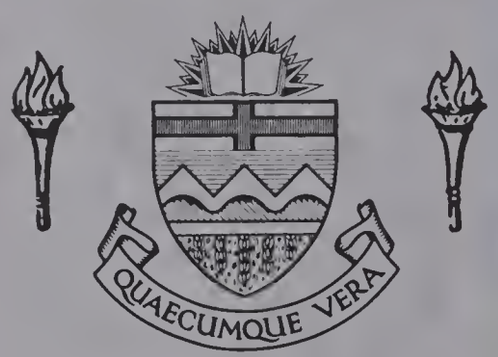


John W. Kimball

BIOLOGY

Third Edition

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Third Edition

JOHN W. KIMBALL

Tufts University



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PREFACE TO THE THIRD EDITION

Ever since I was a child, biology has fascinated me because of the glimpses it gives of what it means to be alive—of how living matter works. This is still what attracts me irresistibly to the subject, and I derive great satisfaction from watching and writing about the continuing rapid progress being made toward understanding the nature of life. And so, in preparing this edition, I have described many of the advances that have been made since the last edition, especially in such areas as how genetic controls work and the operation of the nervous, endocrine, and immune systems.

But today's students have another, equally valid, reason for studying biology. We are now faced in every part of the world with problems that are, at least in part, biological in nature. To attempt to solve these problems (pollution, agricultural productivity, population growth, etc.) requires that we gain the best understanding we can of the biological principles that underlie them. Therefore, in this edition I have not only attempted to deal with some of these problems but have also tried to place the topics alongside the fundamental biology that relates to them. In so doing, it no longer seemed appropriate to sequester "ecology" as a terminal appendage to the book. Instead, I have taken that material, enlarged upon it in many areas (ecosystem productivity, dynamics of soil and water, etc.), and interwoven this material throughout the book.

With new knowledge comes new power. Since the last edition, substantial strides have been made in our understanding of the reproductive process: the determination by somatic cell hybridization of which genes are on which chromosomes, the transfer by reverse transcriptase of genetic information from RNA to DNA, the control of tissue growth by chalones, the direct demonstration of the genetic code, to name a few. But with these fundamental advances, which I have described, have come new possibilities of manipulating the reproductive process. Techniques for isolating genes and introducing them into new cells are advancing rapidly. Sperm banks, embryo transfer, and prenatal diagnosis of genetic disease are already with us. The implications of these technological developments fully justify, I believe, their inclusion in a book of this sort.

A similar tension between new knowledge and the possible applications of it occurs in the area of the responsive systems. Accordingly, I have introduced new material on the processing of information by the central nervous system, on the effect of different drugs on that system, and on a few of the insights that other primates have given us about our own behavior. Included in this section of the text is an entirely new chapter on the immune system which, like the nervous system, learns from experience and helps us to cope with a variety of assaults on the body.

Users of the previous editions will note that the survey of living things that used to begin the book has been reworked in an evolutionary context and placed in the part of the book devoted to evolution. Its former location has now been taken by a new chapter designed to introduce the student to the various ways in which life is studied. This is done through an examination of several facets of a single problem: how atmospheric nitrogen is made available to living things. I hope this chapter will serve as a bridge to the various levels of analysis (including biochemical) that are used later in the book.

Introductory biology texts tend to be compilations of conclusions. For many students, this is a congenial arrangement: they prefer to learn answers and not be concerned with the uncertainties that surround them. It is also the only way to keep a book that tries to survey the entire field to a manageable size. But it is not good science. The pursuit of science has some of the characteristics of a detective story: the gathering of evidence, the weighing of alternative explanations in the light of that evidence, and the testing of alternative hypotheses in new situations. Throughout this edition, I have tried to place greater emphasis on the observations and experiments that form the basis of our present understanding and, where the evidence is weak or contradictory, of alternative hypotheses as well.

I am greatly indebted to many people for their help in the preparation of this edition: to the users of previous editions who took the trouble to give me their suggestions of ways to improve the book; to those who graciously allowed me to reproduce some 80 new photographs and electron micrographs taken from their work; to Ruth Godfrey for typing the manuscript and for her help in keeping my files operating efficiently; to Harper Follansbee and James Funston who read and evaluated the entire manuscript; to Bob Trevor for producing some three dozen new drawings, graphs, and tables; and to the staff at Addison-Wesley for their expert help in every phase of producing this revision.

Andover, Massachusetts J.W.K.
January 1974

PREFACE TO THE SECOND EDITION

The rapidity with which knowledge in biology is advancing dooms any book on the subject to early obsolescence. This edition represents an attempt to narrow the gap momentarily. Literally every section of the book has been reworked in an attempt to bring it more nearly up to date. In particular, the material on the genetic code and gene action has been revised to better reflect the rapid strides being made in that area. The discussion of embryonic development has been rewritten in terms of the selective and sequential unlocking of the genetic code. In this section, the Jacob-Monod theory and its implications for the problems of embryonic development are also examined.

Perhaps the most drastic alteration in the book has been the placing of the material on responsiveness *after* the section on reproduction. This has made possible the rewriting of the chapters on hormones—plant as well as animal—in terms of their action on the transcription and translation of the genetic code.

Among the many other alterations are updated discussions of cell structure and function, nerve and muscle physiology, and new material on the mechanism and control of enzyme action, plant growth and growth regulators, and animal behavior. There are 20 new photographs, photomicrographs, and electron micrographs as well as many new drawings and diagrams.

Material has been deleted as well as added. The survey of organisms in Part II has been made somewhat less formidable by the dropping of some minor groups and the substitution of common names for technical ones in many cases. In fact, the number of technical terms in this edition is substantially lower than in the first.

Many people have assisted with the preparation of this edition. My colleagues and students have been most helpful with their suggestions for improvement. Thanks are due also to the many teachers at colleges and junior colleges across the country who have offered their suggestions and whose acceptance of the first edition has justified the publication of a second. I want to thank also the members of the staff at Addison-Wesley who have worked so hard to make this edition attractive in its appearance.

I am especially grateful to Dr. Jonas Salk and his staff, not only for making their splendid facilities at the Salk Institute for Biological Studies available to me during the preparation of this edition, but for giving so generously of their time and knowledge as well. And, as before, my deep appreciation to my wife, Margaret, for her uncounted hours of typing and proofreading and for her moral support throughout.

La Jolla, California J.W.K.
March 1968

PREFACE TO THE FIRST EDITION

This book grew out of an introductory course in biology for students who were fairly mature and showed promise of being well motivated. However, most of the students in the course had had no prior exposure to biology and consequently the book presupposes none. Those occasional students with a taxonomically oriented course in their background have found that the chapters of Part II provided them with a quick refresher and that the remainder of the book duplicates their earlier course little in content, still less in approach. While some participants in the course have gone on to further work in biology, for many it was their only formal exposure to biology and, in a few cases, to science.

The book is organized along *functional* lines; that is, the various aspects of biology are examined from the point of view of the features and problems shared by all living things. These range from their molecular and cellular organization to their features of metabolism, responsiveness, reproduction, evolution, and ecology. It is hoped that this organization will provide a framework upon which the many facts of biology can be placed in a way that makes sense and makes them easier to remember. Perhaps, too, from it will grow a better appreciation of the unity underlying the diversity of life. Such a system seems to have the advantage of interspersing botanical topics among zoological ones, thus reducing the too-common student antipathy to the former.

Just as a scientist builds upon the work of the past, so students can better understand the significance of the recent discoveries in biology if they first learn the classical statements of the problems. Furthermore, the earlier interpretations are more closely related to what the student can see about him with the aid of the relatively simple tools and techniques of the elementary laboratory. Therefore, the older knowledge is examined first in the chapters. Only then are the discoveries of electron microscopy, biochemistry, and modern physiological techniques discussed.

No attempt has been made in this book to single out just a few areas of biology as illustrative of the materials and techniques of the field as a whole. Most beginning students seem to want the opportunity to see the

whole panorama of the subject. All of the topics of the Advanced Placement Program Syllabus are included as well as a good many others.

One of the most important aspects to teach of any science is the *process* by which knowledge in the field is gained. The interplay of experimentation and reasoning in the growth of science should be made explicit. Unfortunately, to explain the process by which conclusions are reached is far more time-consuming than to describe the conclusions themselves. I have, however, attempted to do it wherever practical. In particular, the chapters on photosynthesis and the work of Mendel are especially designed to impart some appreciation for the process of scientific investigation.

Problems are included at the end of each chapter. These vary from some requiring simply a one-word answer to some for which a sizable essay will have to be outlined. For the student who is interested in pursuing any subject further, additional readings are listed at the end of each chapter. In many cases, the books are available in relatively inexpensive paperback editions. Most of the *Scientific American* articles referred to can be secured in inexpensive reprints from the W. H. Freeman Company of San Francisco. Generally, the references are listed in the same order as that in which the topics they amplify are developed within the chapter.

A great many people have helped me with the preparation of this book. For two years each of my students returned to me—chapter by chapter—his comments and criticisms on the manuscript. In addition, each of the following read the manuscript, in whole or in part, and gave me the benefit of his professional judgment on ways to improve it:

John E. Dowling, Harvard University; Mrs. Frank Eccles, Andover, Mass.; Harper Follansbee, Phillips Academy; Edward Herbert, University of Oregon; Johns Hopkins, III, Harvard University; Herman W. Lewis, National Science Foundation; Alfred Novak, Stephens College; Joseph D. Novak, Purdue University; George K. Sanborn, Phillips Academy; Vincent Silluzio, Newton South High School, Newton, Mass.; Jonathan J. Westfall, University of Georgia; Edward O. Wilson, Harvard University.

Mr. Follansbee deserves special thanks for his constant encouragement and assistance during the years this book was in preparation.

I also wish to thank the many people who so graciously supplied photographs and electron micrographs for the book. Their names appear by their contributions. (The photographs for which no credit is given are my own.) Thanks are due also to Gerard Piel and the editors of *Scientific American,* not only for their helpfulness with this project but also for having done so much to bring new scientific discoveries out of the laboratories and technical journals to the layman. Their efforts have perhaps done more to bring and keep the teaching of introductory biology up to date than any other single force.

The line drawings are the work of William A. Davis. Tirelessly, he worked to match them to the text and to make them not only effective teaching devices but esthetically pleasing as well.

To the staff of Addison-Wesley go my thanks for their cooperation in all aspects of producing the book. And, to my wife, Margaret, my gratitude for the endless hours she spent typing and proofreading version after version, preparing the index and glossary, and for her unfailing support.

All who have participated in the preparation of this book have worked hard to make it as useful and accurate as possible. We hope that its readers will communicate to us any suggestions they may have for its improvement.

Andover, Massachusetts J.W.K.
November 1964

Our planet as seen from the moon by the crew of Apollo 8. (Courtesy of NASA.)

Part I INTRODUCTION



1 THE CHARACTERISTICS OF LIFE

Why Study Biology?

What is Life?

1.1 WHY STUDY BIOLOGY?

One reason stands out above all the others: to learn more about ourselves and the world we live in. Man is an animal. In many ways he differs but little from other animals. In a few ways he differs so greatly as to occupy a unique position in the world. Although it is a debatable point whether man has any attributes not shown to some degree by other animals, it is perfectly clear that he displays some to a far greater degree. One of these is curiosity. *Homo sapiens* is "the man who knows." It is man who always wants to know. Thus we study biology for the same reason we study physics, mathematics, history, literature, and art: to gain knowledge about still another aspect of our lives and our world.

We should also note that productive, rewarding careers can be built on a knowledge of biology. University laboratories are always in need of men and women to make the new discoveries that soon will make this book obsolete. Men and women are also needed to apply their knowledge of biology in such practical fields as medicine and agricultural research. Teachers of biology will continue to be needed to pass the knowledge acquired by earlier generations on to the generations to come.

Biology influences our lives in many ways

Every citizen will be able to participate more effectively in a democracy if he or she can speak out and vote intelligently on questions that involve both biological principles and human welfare. The use of food additives, drugs, insecticides, radiation, techniques of genetic engineering, and population control measures are just a few of the many ways in which our lives may be altered by biological knowledge. Whether this knowledge is used in ways that increase the value of our lives or ways that rob our lives of value can best be decided by informed citizens. To make effective decisions will require not only a clear understanding of the values worth protecting and developing in our lives but also a knowledge of the physical and biological principles that underlie our lives. Knowledge of the first must come from a study of history, religion, philosophy, literature, and art, in other words, the humanities. Knowledge of the second must come from a study of the sciences. This book is an attempt to aid in the study of one of these: biology, the science of life.

1.2 WHAT IS LIFE?

Life is usually easier to recognize than define. We all recognize that a dog is alive, a stone is not (Fig. 1.1). What, then, are the properties of the dog that distinguish it from the stone?

1. The Complex Organization of Life

Some stones may seem to be rather complex with various minerals scattered through them. Their organization is simplicity itself, however, when contrasted with that of a dog. If we examine any part of a dog's body with a microscope, we shall discover that it is made of **cells.** These units, generally too small to be seen with the unaided eye, are organized into



Fig. 1.1

Living things must be studied at many levels of structural organization

tissues which, in turn, form **organs** such as the stomach and kidney. Several organs, e.g. the stomach, liver, and intestine, work together as a **system.**

Biologists have studied these levels of the organization of life for many years. More recently they have been able to probe still deeper into the complexity of living things. The electron microscope has revealed a degree of subcellular structure and organization that twenty years ago was hardly suspected. Physical and chemical tools and techniques are now enabling us to dissect these subcellular structures into the molecules and atoms of which they are made.

Rocks are made of atoms, too, but never in such intricate patterns or with such variety. Furthermore, the atoms of a rock are more rigid in their organization. The rock we see today contains the same atoms it contained last year. The dog we see today is largely constructed of atoms acquired since we saw him last year. Those he has lost have returned to the environment. This rapid turnover of material in living things is part of their **metabolism.**

2. Metabolism

A dog continually exchanges material with its surroundings. We see it eat and drink. It defecates and urinates. It breathes. With proper equipment, we can show that the air the dog exhales differs in composition from the air inhaled: oxygen has been removed and carbon dioxide added.

A living organism continuously exchanges matter with its surroundings

All organisms share this property with the dog. Whether rapidly or slowly, they all take in material from and give material back to the environment. Furthermore, the material given back is not the same as the material taken in. We have seen that exhaled air differs from inhaled air. Many substances found in fecal matter and urine were not present in the food that was eaten. During their relatively brief stay within the dog's body, food materials undergo extensive transformations. As they do so, energy is liberated. Ultimately this energy appears as heat and warms the dog. But before this occurs, the energy may be used for some of the other activities characteristic of life.

3. Reproduction

A living organism acquires matter and assembles it into its own characteristic patterns

The essence of reproduction in a living organism is the self-controlled duplication of the structures characteristic of it. It occurs when the organism takes in more material from its environment than it gives back and organizes these materials into its own structures. We call this kind of reproductive activity **growth.**

To do this, the organism must expend some of the energy released during its metabolism, and it must have a pattern that will guide the building of its structures. If you have studied chemistry, you might argue that the crystals in a rock, once at least, grew in the same way. A crystal placed in a solution of its units does slowly organize these units according to its pattern and thus grows. There is one basic difference, however, between growth of a crystal and growth of a living organism. The crystal grows by accumulating from its surroundings units that are already identical with those in the crystal. The living organism, on the other hand, grows by transforming materials that are not unique to it into those that are. Our dog can thrive on the same diet that we eat. He, however, converts this diet into more "dog"; we convert it into more "human."

Reproduction is the formation of new individuals

Reproduction also involves the production, from time to time, of copies of the organism that can live independently of it. All living things must die sometime and, if their kind is to survive, they must make copies of themselves before they die.

Among plants and the less complex animals, this aspect of reproduction may simply be an extension of the growth process. The growing strawberry plant sends out horizontal stems on which develop "daughter" plants. This kind of reproduction is called **asexual** because only one parent is involved. Generally, the daughter plants are identical with the parent.

Asexual reproduction preserves the status quo

Almost all organisms (including dogs) engage in another kind of reproduction. **Sexual** reproduction requires that *two* parents contribute to the formation of the new individual. In this way, new combinations of



Fig. 1.2 *Neopilina.* This primitive mollusk was discovered in 1952. It differs little from its closest relative, *Pilina,* which has been extinct for 350 million years. (From the *Galathea* Report.)

Sexual reproduction produces new combinations of traits

traits can be produced. This is apt to involve greater complexities than asexual reproduction, as the two parents must locate each other and, in many cases, be equipped to care for their offspring until the offspring can care for themselves.

4. Responsiveness

All living things are capable of responding to certain changes (**stimuli**) in their surroundings. Changes in light, heat, gravity, sound, mechanical contact, and chemicals in the surroundings are common stimuli to which living organisms respond. In order to respond to these stimuli, the organism must have a means of detecting them. The eyes, ears, and nose of the dog are effective stimulus detectors, as anyone who has watched these animals can testify.

In order to be effective, responses to changes in the environment must be coordinated. Even the simplest organisms consist of many parts and each of these must do the right thing at the right time for an appropriate action to be carried out. When our dog is called to supper, some of his muscles must contract, others must relax, his digestive glands must begin to function and so on. Each part must work in harmony with the others.

Animals coordinate the activities of their organs by nerves and hormones

A system of **nerves** and a system of chemical regulators called **hormones** coordinate the actions of the dog and many other animals. Plants rely on hormones for their coordination.

The action carried out by an organism in response to a stimulus is accomplished by its **effectors.** Muscles and glands are the most important ones in animals. Like all other parts of the coordinating system, they consume energy. Energy enables the dog's muscles to contract and his glands to synthesize the enzymes with which he will digest his supper.

Organisms respond to changes in their environment by altering their relationship to it. In running to his supper dish, the dog is changing his position in response to your signal. These responses, which often occur in definite patterns, make up the organism's **behavior.** It is an active, not a

passive, thing. A dislodged stone rolling downhill is not behaving; it is simply being carried along passively by the force of gravity. Our hungry dog, on the other hand, is *creating* the change in his relationship to his surroundings.

5. Evolution

When organisms reproduce themselves, their pattern is copied with marvelous accuracy. Dogs have puppies, not kittens. Children sometimes share distinctive traits with their mother or father. The little marine animal *Neopilina,* which was dredged up from the bottom of the Pacific in 1952, looks practically identical with its ancestors preserved as fossils over 500 million years ago (Fig. 1.2).

Evolution is the gradual change in the characteristics of a species over the course of successive generations

Neopilina is not, however, identical with its ancestors. Nor are any other organisms found on earth today identical with those that lived in ages past. Over long periods of time, changes have occurred. These changes mark the evolution of the organisms. Evolution has often been adaptive; that is, the changes have enabled the organisms to live in their environment—to metabolize, reproduce, and respond—more efficiently than their ancestors could have in the same environment. The many breeds of dogs testify to the ability of organisms to evolve over time, although many of the changes which man has wrought in dog evolution can hardly be thought of as adaptive.

Evolution is also the formation of two or more species from one ancestral species

Evolution involves something else. The number of kinds of organisms that now live on the earth is far greater than the number that were present 500 million years ago. From that time to this, there has been a proliferation of kinds of organisms. This aspect of evolution has occurred as single groups of organisms have given rise to two or more distinct kinds of descendants. All our present dogs, for example, are probably descendants of one ancestral type.

These then are the major characteristics of living things. Although all share them, how they accomplish or express them differs to some extent from one organism to another. The remainder of this book will be devoted to examining how representative organisms accomplish each of the major activities of life.

EXERCISES AND PROBLEMS

1. Does a candle flame show any of the characteristics of life?
2. In what ways are growth and reproduction similar? In what ways are they different?
3. How is metabolism related to growth?
4. How is responsiveness related to metabolism?
5. What are the differences between sexual and asexual reproduction?
6. Can most animals reproduce asexually? Can most plants?
7. What is a stimulus?
8. In what ways have discoveries in biology affected your life?

2 THE STUDY OF LIFE

Introduction

The Problem of Nitrogen Fixation

Early Experiments

Symbiosis and its Establishment

Specialization and the Division of Labor

The Global Implications of Nitrogen Fixation

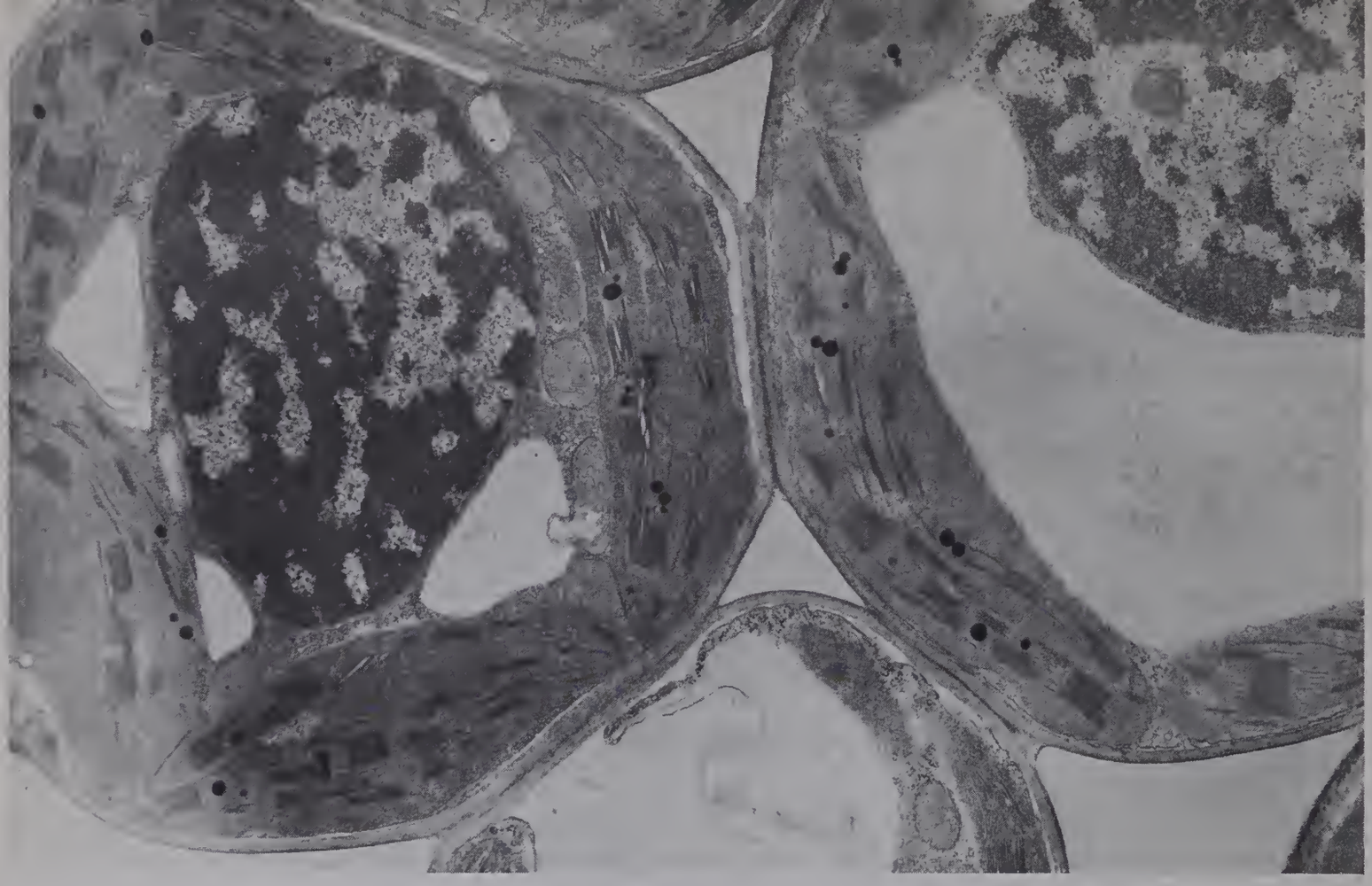


Fig. 2.1 Leaf cells of the sunflower as seen under the electron microscope. Portions of four adjacent cells are shown. Note how the interior of each cell is made up of discrete components (organelles) which in themselves show considerable structural complexity. Structure which can only be revealed by the electron microscope is called ultrastructure. (Courtesy of H. J. Arnott and K. M. Smith.)

2.1 INTRODUCTION

The cell is the smallest structural unit of an organism that has all the attributes of life

The study of life is as varied and complex as life itself. No one investigator, no single approach can possibly unlock all its secrets. The analysis of living matter must go on at a number of levels of complexity. We can investigate its structural organization at the level of the individual cell—often called the unit of life—but this by no means answers all our questions. Cells contain discrete subcellular structures ("organelles") such as the nucleus (Fig. 2.1). These are, in turn, constructed from giant molecules such as proteins, carbohydrates, and nucleic acids. The molecules themselves are made up of atoms. Atoms have structures and although the study of atomic structure is usually thought to be the job of chemists and physicists, many problems in biology cannot be solved without this knowledge.

While the cell represents the highest level of organization for some creatures (e.g. an amoeba or yeast—Fig. 2.2), larger organisms use cells as building blocks with which they achieve even more elaborate levels of organization (Fig. 2.3). But even if we could describe completely the organization of a creature as complex as man, we would not understand all

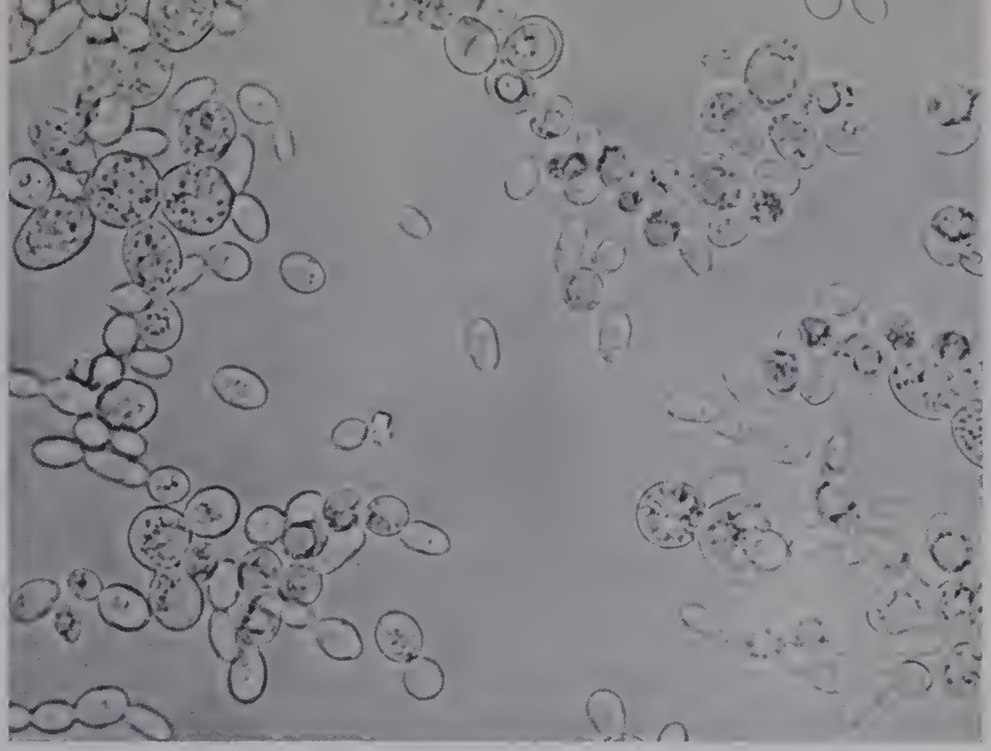


Fig. 2.2 Yeast cells growing in culture. Note the many cells that are in the process of reproducing.



Fig. 2.3 *Oscillatoria,* a filamentous blue-green alga (1000 X). Each of the disks in the chains is one cell.



Fig. 2.4 Left: a common rock lichen. Right: its structural organization.

there is to life. Organisms live side-by-side with other organisms of their own kind as well as with organisms of different kinds. Often these interactions occur in characteristic patterns both structural and functional. A school of fish, a beehive, the fungus and alga that together make up a lichen (Fig. 2.4), and the mutual dependence between flowers and the animals that pollinate them are examples.

Morphology is the study of structure

At each level of organization, an investigator may choose to focus on its structure, that is, the form in which its parts are assembled, or he may choose to study the *interactions* between various structural components. The study of structure is called **morphology,** and most of the early successes in biology came from its study. The history of biology is marked by a succession of keen observers who dissected and described the structure of plants and animals, their organs and tissues, and—as the necessary tools were developed—their cells and the molecules out of which *these* are made.

Physiology is the study of function

The study of the interactions between structural components, that is, how things *work,* is called **physiology.** Physiological studies have tended to lag behind morphological ones, because so much physiological analysis has depended on the success of chemists and physicists in devising equipment and procedures for the physiologist to use. Nevertheless, we find ourselves today swept along in a rising tide of physiological discoveries that have gone a long way toward answering questions as to how the components of living matter—at every level of organization—interact with one another. Life can only be understood if we understand the interactions of its constituent atoms, molecules, cell organelles, cells, tissues, organs, and systems. We must also try to describe the mechanisms by which members of the same species (e.g. honeybees) interact with each other and the mechanisms by which *different* species interact (e.g. eating and avoiding being eaten). The tools and techniques are also being developed by which we can better assess the interactions between entire populations of creatures and the environment—both living and nonliving—that surrounds them. The space explorations of the 1960's, coupled with rapid population growth and earthbound technological development, have helped to awaken us to a keen awareness of how thin and fragile is the layer of air,

Interactions occur between the structural units of living matter at every level of organization

soil, and water on the surface of our planet upon which we and all other creatures depend for our existence.

The remaining chapters of this book will examine life—both its morphology and physiology—at each of the levels of complexity we have been discussing. Before we begin, however, let us turn to the examination of a *single* biological problem. In so doing, perhaps you will gain an appreciation of the value of a diversity of approaches and techniques in attempting to solve biological problems. The problem is how living creatures secure nitrogen.

2.2 THE PROBLEM OF NITROGEN FIXATION

Nitrogen is essential to all life

Nitrogen is an absolutely indispensable component of all life on this earth. No biological structure could be built without the participation of proteins and nucleic acids, both of which contain large amounts of nitrogen. The problem of securing nitrogen is by no means of theoretical interest only. With protein malnutrition so widespread in the world—and at a time when the human population is rapidly increasing—an understanding of the way creatures secure nitrogen assumes enormous practical importance.

Since four-fifths of all the air we breathe is pure nitrogen, you might well wonder why there is any problem. But pure nitrogen (N_2) is a very inert substance. With all our technological achievements, we have never found a way to "fix" nitrogen, that is, to get nitrogen to combine with other atoms (and thus provide the building blocks of living matter) except at extremely high pressure and temperature. And when we do this, we are simply duplicating the natural process by which lightning bolts provide the energy to force some nitrogen to combine with oxygen atoms in the air ($N_2 + 2O_2 \longrightarrow 2NO_2$). It has been estimated that seventy lightning bolts strike somewhere on earth every second, but even so, the amount of nitrogen fixed by lightning probably meets less than 10% of the needs of the earth's creatures.

Nitrogen atoms do not combine readily with other kinds of atoms

Nitrogen fixation is the conversion of atmospheric nitrogen (N_2) into nitrogen-containing compounds

Where does the rest come from? A number of different organisms are able to pull off the trick we humans cannot. They are able, under the conditions existing within their cells, to take atmospheric nitrogen and fix it into compound form (e.g. ammonia, NH_3). Fixed nitrogen can then serve as the starting material for the synthesis of proteins and nucleic acids.

The ancient Greeks and Romans knew that continued cultivation of some crops made the soil less fertile, while the cultivation of plants of the family we know as legumes improved the fertility of the soil. Peas, beans (including soybeans), clover, and alfalfa are all legumes that not only provide nutrition for man and beast but at the same time improve the soil they grow in. The Roman, Columella, writing in the first century A.D. explained that "of those [fodder plants] which find favor the medic plant [alfalfa] is outstanding for several reasons: one seeding affords, for all of ten years thereafter, four harvestings regularly and sometimes six; it dungs the land; and lean cattle of every kind grow fat on it." The Romans also

Legumes are pod-bearing plants of the family that includes peas and beans

knew that if leguminous crops were not harvested but instead plowed into the soil, the beneficial effect on the soil was further enhanced.

Several minerals (e.g. saltpeter, KNO_3) when mixed into soil provide the same stimulating effect on plant growth that legumes do. With the discovery of nitrogen late in the seventeenth century, it soon became apparent that it was the nitrogen that these minerals contained that was the important ingredient. With the development of techniques for measuring how much nitrogen (in compound form) was present in such materials as soil and plant parts, it then became possible to see what role legumes play in the nitrogen economy of the soil.

2.3 EARLY EXPERIMENTS

Boussingault demonstrated that nitrogen fixation is increased where legumes are grown

Early in the nineteenth century, Jean Baptiste Boussingault, a prominent French chemist, started the first agricultural experiment station on his farm in Alsace. Over a period of many years he planted a succession of leguminous and nonleguminous crops in a number of experimental plots. Each season he determined the amount of fixed nitrogen added to the crop (in the form of manure) and the amount of fixed nitrogen present in the crop at harvest. As the table in Fig. 2.5 shows, in those plots where at least one crop of a legume (clover) had been planted, there was a small increase in the amount of fixed nitrogen (averaging 11 lb per acre per year). Where the legume alfalfa had been planted *every* year, however, some 130 lb of nitrogen was fixed in each acre each year.

Boussingault's Results

Year						Nitrogen		
1	2	3	4	5	6	In manure	In crop	Total gain
Potatoes	Wheat	Clover	Wheat	Turnips		185	229	44
Mangel	Wheat	Clover	Wheat [1]	Oats		185	231	46
Potatoes	Wheat	Clover	Wheat [1]	Peas	Rye	223	323	100
Fallow	Wheat	Wheat				76	80	4
Lucerne [2]	Lucerne	Lucerne	Lucerne	Lucerne	Lucerne	205	980	775

(Figures are pounds per acre)

[1] Wheat followed by turnips the fourth year.

[2] A legume (alfalfa).

Fig. 2.5 Results of Boussingault's field studies of nitrogen fixation.

In an effort to improve the precision of his experiments, Boussingault then undertook to grow legumes in sterilized sand in closed containers. In this way he hoped to be able to show that atmospheric nitrogen was taken up by the plants and fixed in their own tissues. Unfortunately, no such thing occurred. The plants grew poorly and there was no evidence of nitrogen fixation. Boussingault wrongly concluded that all of his earlier findings had been in error.

The problem was the sterile soil. In 1886, two German scientists, Hellriegel and Wilfarth, grew peas in (1) sterile sand, (2) nonsterile sand, (3) sterile sand to which an extract of rich garden soil had been added. A nitrogen-containing fertilizer was added to some, but not all, of the pots in *each* of the three groups. The surface of the pea seeds was sterilized before planting and all watering was done with sterile water.

All plants receiving fertilizer nitrogen grew luxuriantly (Fig. 2.6). Without fertilizer, however, only the plants in group 3 consistently grew well. *Some* of the plants in group 2 grew well and some did not. Those that did not were stunted and their leaves yellow instead of dark green. *All* the plants in group 1 were stunted and yellow.

	Sterile sand	Nonsterile sand	Sterile sand + soil extract
Fixed nitrogen fertilizer added	+	+	+
No fixed nitrogen added	−	±	+

\+ Luxuriant growth
− Stunted growth
± Some plants luxuriant, some stunted

Fig. 2.6 Results of the experiments of Hellriegel and Wilfarth.

Legumes can only fix nitrogen if their roots have nodules

When the plants in the groups that had not received fertilizer were removed from their pots, an interesting correlation was seen. The roots of all the plants that had grown well were covered with small nodules (Fig. 2.7). None of the stunted plants had nodules. Evidently something regularly found in garden soil, sometimes found in nonsterile sand, but never found in sterile sand had (1) caused nodules to appear on the pea roots and (2) enabled the plants to grow luxuriantly without added fertilizer.

Rhizobia are bacteria that infect legume roots

That something turned out to be tiny single-cell organisms called bacteria. The particular bacteria that were producing the effect are now called rhizobia. They are often found in soil and are especially abundant in soil where legumes have been grown.

2.4 SYMBIOSIS AND ITS ESTABLISHMENT

How do these bacteria enable legumes to fix atmospheric nitrogen? The answer is not fully known even today, although much new information has been discovered since the experiments of Hellriegel and Wilfarth. In order to do the job, the bacteria must actually enter the tissues and even the individual cells of the legume roots. The discovery of how this is done has required a number of analytical approaches. Histologists are scientists who study the morphology of tissues. By preserving root tissue at all stages of nodule formation, and by preparing thin slices of these tissues and



Fig. 2.7 Nodules on the roots of the birdsfoot trefoil, a legume. (Courtesy of The Nitragin Co., Milwaukee, Wisconsin.)

staining them with dyes, they make specimens that can be examined under the light microscope. More recently, analogous techniques have been used to follow the process under the higher magnifications of the electron microscope. These studies have shown that the bacteria enter a surface cell of the root and then migrate into the interior of the root, moving along an intracellular channel that grows through one root cell after another (Fig. 2.8). This channel (the "infection thread") is actually constructed by the root cells, not the bacteria, and is formed only in response to the infection. When the infection thread reaches a special cell in the interior of the root, it bursts and the bacteria swarm through the cell. After a period of multiplication, they begin to change shape and lose their motility. These bacteroids, as they are now called, may almost fill the cell (Fig. 2.9). Only at this time does the plant begin to fix atmospheric nitrogen.

Formation of the infection thread

Once rhizobia are released within a root cell, they become transformed into bacteroids

Other kinds of bacteria may invade plant roots. In so doing, they cause disease just as many bacteria that invade our tissues do. We describe such invasions as infections. But here is an infection that brings great benefit to the host and (as we shall see) to the bacteria as well. We use the term **symbiosis** to describe a prolonged, intimate relationship between two different kinds of organisms.

Symbiosis

The physiology of the infectious process has also received study. It has been shown that legume roots secrete into the soil a substance that attracts rhizobia. On the other hand, the bacteria synthesize and secrete substances —hormones—that are normally found only in plants. It is perhaps through

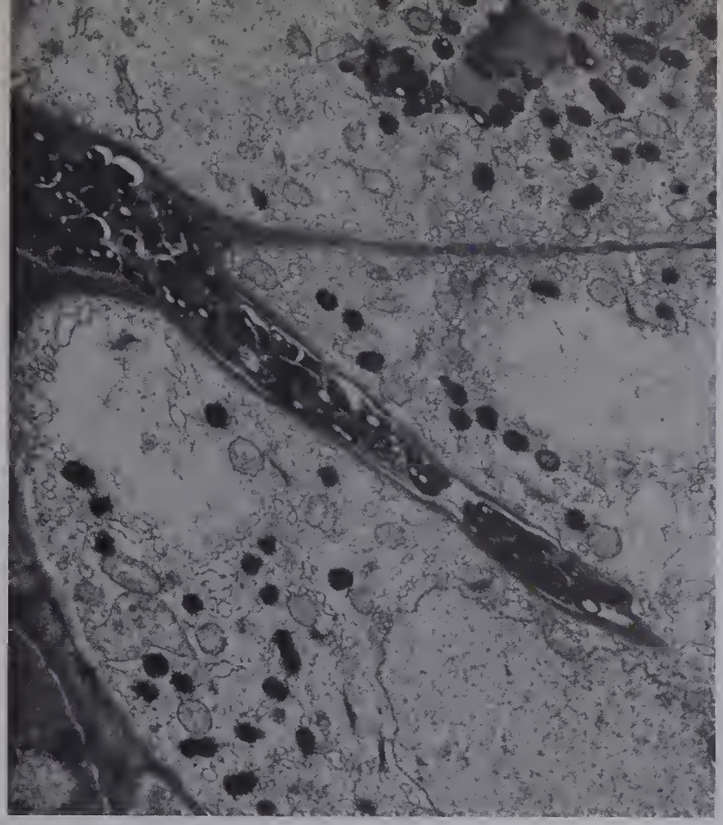


Fig. 2.8 Rhizobia-filled infection thread growing into a cell (from upper left to lower right). Note how the wall of the infection thread is continuous with the wall of the cell. Once the thread ruptures, rhizobia are released directly into the cytoplasm of the cell (dark ovals). (Electron micrograph courtesy of Dr. D. C. Jordan.)

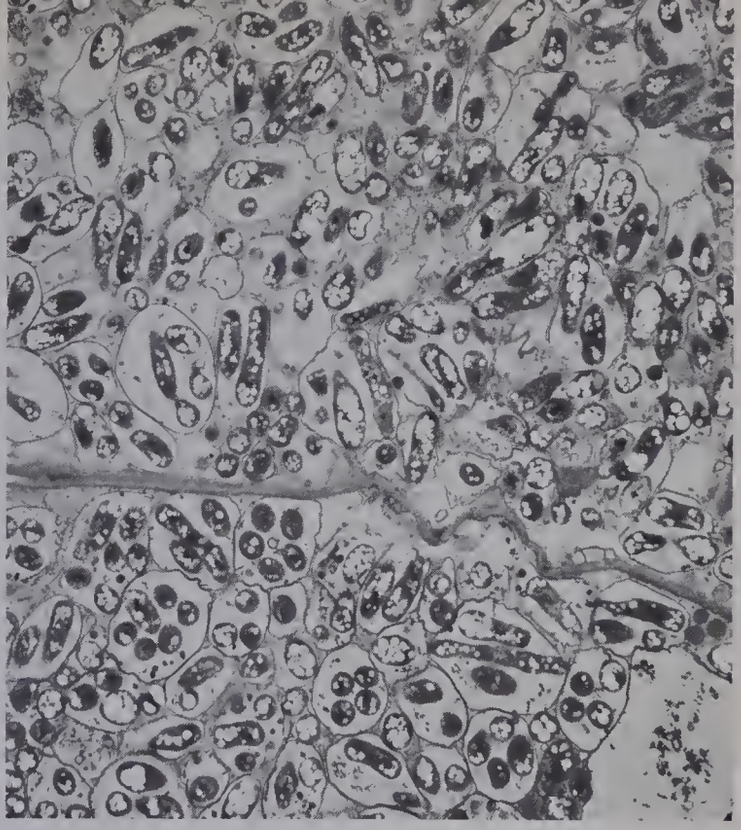


Fig. 2.9 Bacteroid-filled cells from an active soybean nodule. The horizontal line marks the walls between two adjacent nodule cells. (5000 X, courtesy of R. R. Hebert.)

this action that they are able to get the plant to alter its normal pattern of root growth and cause nodules to be formed.

Nodules are constructed by the legume, not by the rhizobia

Nodules are not simply structureless masses of bacteroid-filled cells. While these cells are present and are the site of nitrogen fixation, other plant tissues—of perfectly normal appearance—participate in the formation of the nodule (Fig. 2.10). These include a system of conducting vessels or ducts which connect with the other ducts of the plant. Through these ducts, nutrients pass to and from the nodule and the rest of the plant.

Thus the development of the nodule, while dependent on the presence of the bacteria, is guided by the normal control mechanisms of the plant. The information (encoded in genes) for making nodule tissue resides in the plant, not in the bacteria. The expression of this information in the development of a nodule is, then, a special case of the expression of genetic information in the building of normal root tissues and, indeed, in the building of the entire plant.

Biologists whose main concern is how a seed, for example, grows into a mature plant or how a fertilized egg grows into a human, are called developmental biologists (or embryologists).The events of nodule formation are also problems for the developmental biologist to investigate. In all

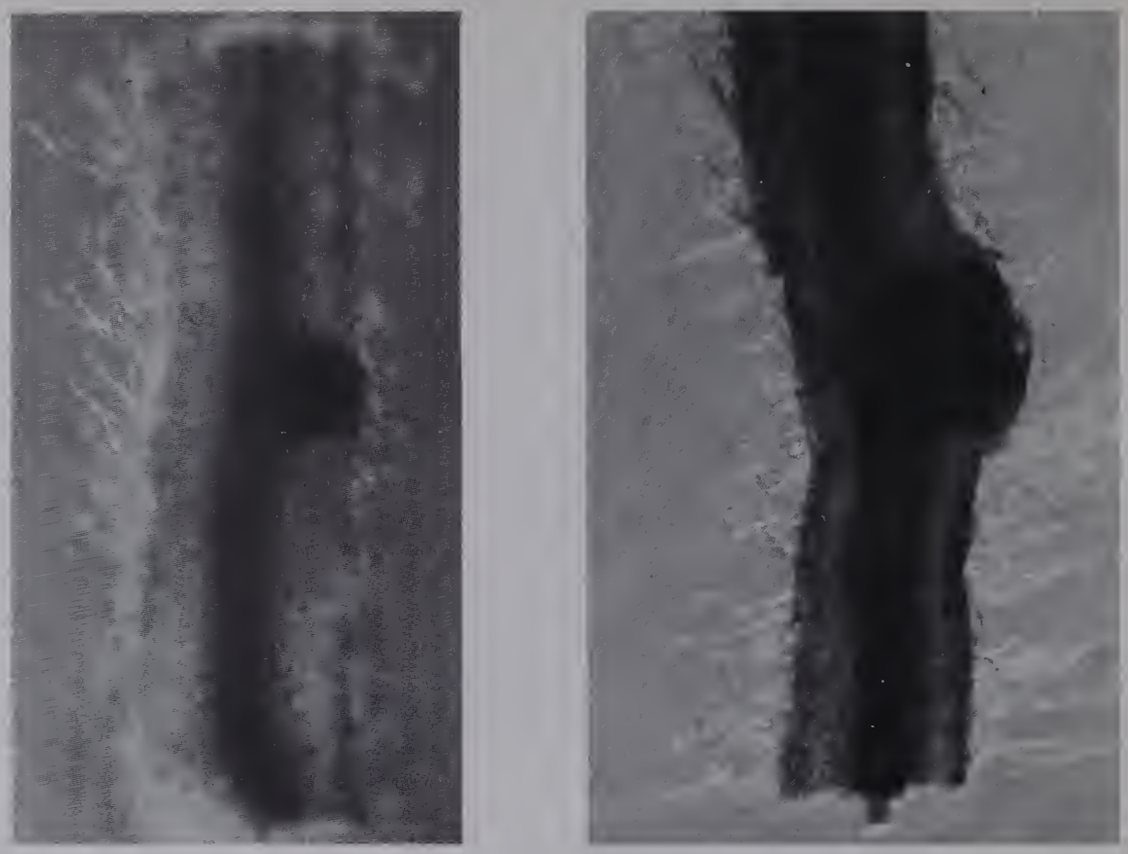


Fig. 2.10 Left: segment of a pea root showing a developing lateral root. Right: segment of pea root showing a developing nodule (12 days after the root was infected by rhizobia). Both structures are connected to the nutrient transport system of the plant (dark area extending through the center of the root). Photomicrographs 10X, courtesy of John G. Torrey.

these cases, the same question is asked: how is coded information expressed so as to give rise to characteristic structures and functions? We shall examine later in the book some of the hard-won scraps of knowledge of how patterns of growth unfold in the developing organism.

There are many strains of rhizobia, each differing in the effectiveness with which it can live symbiotically with any particular legume

Although all rhizobia look alike, they do not act alike. Some will infect only peas, some only clover, some only alfalfa, etc. The geneticist, an investigator of heredity, would like to know what genes (in the plant or in the bacteria or both) control the ability of rhizobia and legumes to establish a successful symbiosis. In the meantime, bacteriologists work to isolate and cultivate the various strains of rhizobia so that the most efficient types can be made available to farmers. The deliberate exposure of legume seeds to the proper rhizobia is now a routine agricultural practice. The photograph in Fig. 2.7 was taken by research workers in a company that specializes in producing rhizobial strains appropriate to each leguminous crop.

2.5 SPECIALIZATION AND THE DIVISION OF LABOR

Without rhizobia, legumes cannot fix nitrogen. But without legumes, rhizobia can't do it either. This is so, even though a number of other kinds

Some soil bacteria fix nitrogen by themselves

of soil bacteria are able by themselves to fix nitrogen, having no need to establish any sort of symbiotic relationship with another organism. Clearly, legumes and rhizobia are mutually interdependent with respect to their ability to fix nitrogen. How might one go about trying to find out what essential ingredients each supplies to the process?

Isolated bacteroids can fix nitrogen

The classic approach to a problem of this sort is to separate the parts of the system, try to find out what each does, and then try to recombine them in the "test tube" into a functioning unit. Nodule cells can be broken and the bacteroids isolated. From the bacteroids can be extracted *all* the materials needed for nitrogen fixation.

Then why is the legume necessary? The legume is certainly *helpful* in that it supplies nutrients to the bacteroids from which they secure the large amounts of energy needed to force the inert molecules of nitrogen into compound form.

In order to secure energy from their food, the bacteroids also need oxygen (just as we do). However, the process of nitrogen fixation itself is strongly inhibited by the presence of oxygen. Thus the bacteroids must walk a metabolic tightrope between too much and too little oxygen. Their job is probably made easier by a second contribution from their host: hemoglobin. Hemoglobin is the red pigment that transports oxygen in our blood. It seems to perform a similar function in the cells of the nodule (to which it is confined): releasing just enough oxygen to the bacteroids (which are literally immersed in it) to satisfy their conflicting requirements.

The hemoglobin in nodule cells is synthesized by the plant, not by the bacteroids

Beyond these contributions, though, the plant must also—in a way as yet unknown—make possible the conversion of rhizobia that cannot fix nitrogen into bacteroids that can.

Although the sequence of steps by which nitrogen fixation occurs is still under active study, it is now quite certain that the first stable product formed is ammonia, a simple compound of nitrogen and hydrogen (NH_3). From ammonia, all the other nitrogen-containing substances of the plant (e.g. proteins, nucleic acids) can be synthesized. The study of the chemistry of nitrogen fixation is an example of **biochemistry:** the study of all the chemical interactions in living things.

The first stable product of nitrogen fixation is ammonia

How did two such different organisms ever work out such an intimate and complex living relationship? Or, to put it as an evolutionary biologist might, how did this symbiotic relationship *evolve?* Here, too, the answers are only tentative. Assuming that the ancestors of the rhizobia could carry out the entire process themselves—as many other soil bacteria still do—they must have gained some real advantage from learning to share the duties with a legume. Perhaps the environment provided by the host, e.g. lots of food and just the right amount of oxygen, enables rhizobia to do the job more efficiently than before and *also* to stop carrying out inefficiently certain functions that the legume can carry out more efficiently. Later on we shall see other examples, at several levels of organization, of the increased efficiency that specialization brings and the accompanying loss of independence that is the price that must be paid.

The evolution of symbiotic nitrogen fixation



Fig. 2.11 Demonstration that clover grows normally only where the supply of molybdenum is adequate. The soil shown here (in Eastern Australia) is naturally deficient in molybdenum. Although the entire fenced-in plot was seeded to clover, the plant was able to flourish and fix nitrogen only where molybdenum fertilizer had been added. (Courtesy of A. J. Anderson.)

2.6 THE GLOBAL IMPLICATIONS OF NITROGEN FIXATION

How can we exploit this symbiotic relationship to help feed a hungry world? This requires the help of geneticists and bacteriologists to provide the most effective symbiotic combinations. It also requires the efforts of plant physiologists to determine the best conditions of soil temperature, moisture, acidity, and mineral content for maximum nitrogen fixation. They have found, for example, that the use of nitrogen-containing fertilizers suppresses nitrogen fixation. On the other hand, they have found that certain minerals *must* be present in the soil for nitrogen fixation to be successful. One of these is molybdenum. As little as one ounce of molyb-



Fig. 2.12 An algal bloom in Lake Minnetonka, Minnesota. Drainage into the lake of nutrients from cesspools, sewage treatment plants, and fertilized agricultural land has supported this explosive growth of algae. (Courtesy of the Minneapolis Star.)

Molybdenum is essential for nitrogen fixation

denum can convert an acre of infertile cropland in Australia into land capable of supporting luxuriant crops of nitrogen-fixing clover for up to ten years (Fig. 2.11).

We have alluded to the fact that some soil bacteria can fix nitrogen without the help of legumes. Thus they (unlike rhizobia) must possess all the genetic information needed to carry out the job. The implications of genetic engineering of which you may have heard (and which we shall discuss in Chapter 19) have not escaped research workers interested in nitrogen fixation. In 1972, two scientists in England, R. A. Dixon and J. R. Postgate, succeeded in transferring the genes required for nitrogen fixation into the cells of the bacterium, *E. coli* (which grows by the billions in our intestines). While this maneuver probably will not enable us to give up our dependence on plants and animals for our nitrogen needs, it does raise the possibility of inserting these genes into other kinds of cells. The major sources of energy for the human species are the cereal grains: rice, wheat, and corn. While these are superb at meeting our calorie requirements, the protein they produce (and for which they need large amounts of fixed-nitrogen fertilizer) is inadequate to meet human dietary needs. If functioning nitrogen-fixation genes could be introduced into these plants, that would represent an extraordinary advance in the ability of the human species to feed itself.

Could nonleguminous plants be given the genes to enable them to fix nitrogen?

Agriculture and industrial technology may account for half the nitrogen fixed on our planet

But there are other aspects to nitrogen fixation that we ignore at our peril. It is estimated that at the present time man's activities account for almost 50% of all the nitrogen fixation that occurs on earth. The bulk of this is through industrial fixation (mainly providing fertilizers) and the

ever-increasing planting of leguminous crops. We shall examine in Chapter 11 the way in which fixed nitrogen gets back to the atmosphere as N_2. But it is very likely that this return process is not keeping up with the accelerating rate at which we are fixing nitrogen. If we are adding increasing amounts of fixed nitrogen to the environment with every passing year, what are the implications?

They are already to be seen. In aquatic environments (e.g. a lake) the amount of fixed nitrogen affects the amount of algae and other life that can grow there. In many parts of the world, especially in densely populated regions or where intensive, heavily fertilized agriculture is practiced, increasing amounts of nitrogen are reaching water supplies. The result is the periodic, often explosive growth ("blooms") of algae that make the water unsatisfactory for drinking, recreation, and other uses (Fig. 2.12).

Algal blooms

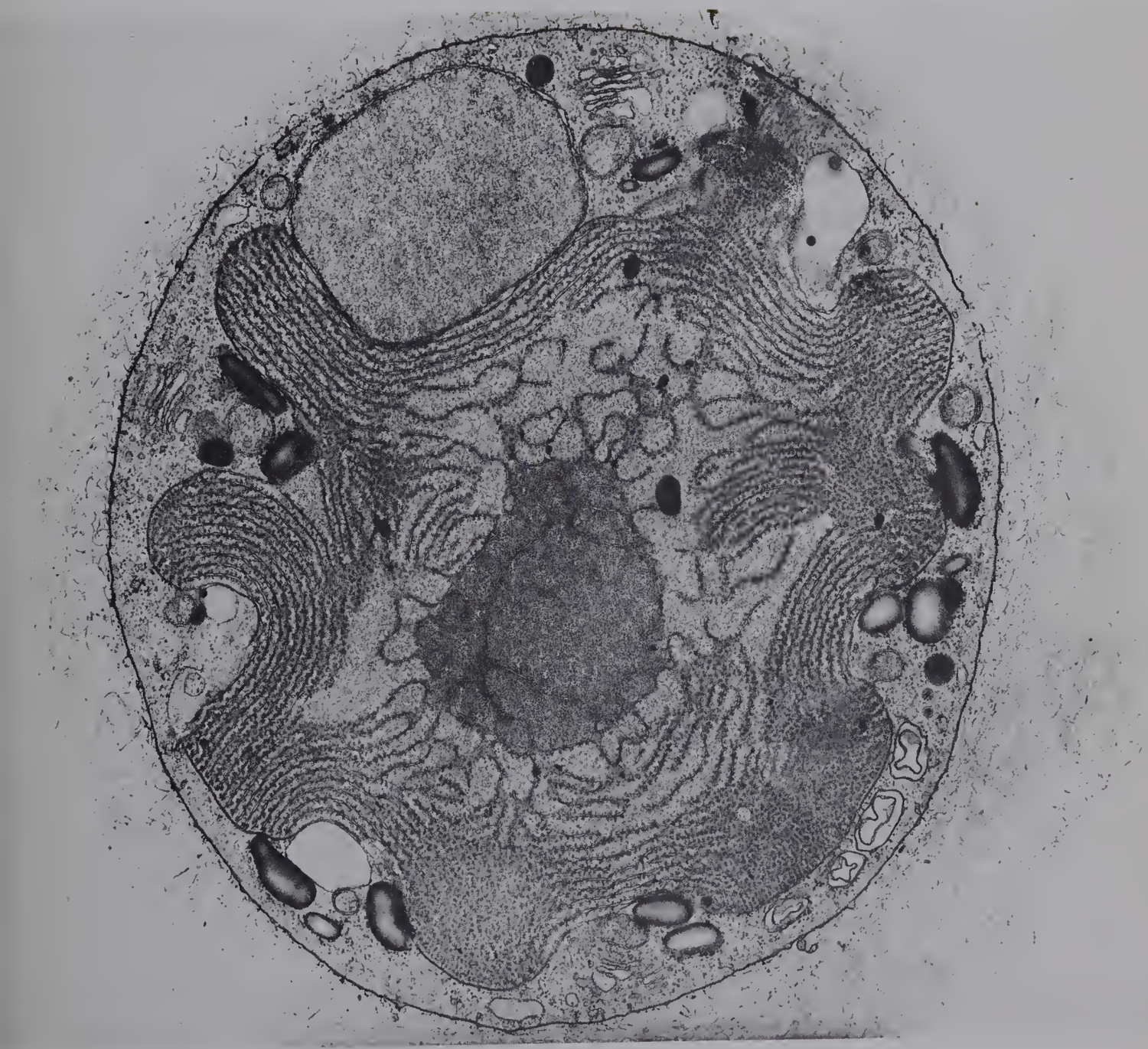
The problem of nitrogen fixation is, then, one of great theoretical and practical importance. It raises fundamental questions of biochemistry, physiology, and developmental biology. It raises important questions about evolutionary mechanisms and evolutionary history. It raises troublesome questions about the balance of nature, i.e. about ecology. We have examined some of these questions not only for their own intrinsic interest but also to illustrate the variety of approaches that can be used to explore the nature of life.

REFERENCES

1. Delwiche, C. C., "The Nitrogen Cycle," *Scientific American,* Offprint No. 1194, September, 1970.

Porphyridium, a unicellular microorganism. The electron microscope reveals a wealth of structural details within its single cell. (18,000 X, courtesy of E.Gantt.)

Part II THE ORGANIZATION OF LIFE



3 THE CHEMICAL BASIS OF LIFE: PRINCIPLES

Elements

Atoms

Chemical Compounds

Molecules

Formulas

Ionic Compounds

Acids

Chemical Changes

Types of Chemical Change

Rate of Chemical Reaction

Catalysis

Reversible Reactions

Mixtures

Life occurs on our planet in an almost unbelievable array of forms. For many years, biologists were fully occupied with the discovery, the classification, and the anatomical study of the many species of living things. However, as more and more was learned from the study of energy (physics) and the study of matter (chemistry), it became possible to analyze living things in terms of structural units far smaller than even those visible under the microscope. These units are atoms and molecules.

As a result of such studies, two things have become clear.

1. Although living things are diverse in their appearance, habitat, etc., their basic chemical organization is remarkably similar.

2. Although the chemical organization of living matter is very complex, it is based upon the same materials and principles as those found in the world of nonliving things.

These two discoveries have had far-reaching consequences. They have shifted the attention of biologists from the many ways in which living things differ from one another to the many ways in which they are similar. This shift in approach has gone hand in hand with an enlargement of our way of looking at living things. No longer can a biologist be content simply to study morphology, that is, the way in which organisms are put together out of cells, tissues, and organs. Now he must ask how these various structural parts of the organism work; that is, he must also examine their physiology. To find the answers he must understand the chemical makeup of the living cell and the chemical principles underlying its activity.

3.1 ELEMENTS

All the things found on this planet—air, sea water, soil, rocks, etc.—are composed of one or more fundamental building blocks of matter, the elements. Despite the large number of kinds of rocks, minerals, etc., only 90 different elements have been found occurring naturally on earth. Actually, at least 105 different elements are known, but 15 of these have been man-made in the laboratories of nuclear physics.

We can define an element as a substance that cannot be further decomposed by chemical means. Iron, oxygen, aluminum, and silicon are four common elements found on or near the surface of the earth. To say that everything on this earth is composed of, and is thus reducible to, one or more of 90 different elements does not exclude living things. They, too, are made up of elements. Of the 90 present generally on earth, however, only about one-quarter are found in living organisms. Not only is the number of elements found in living organisms smaller, but the relative prevalence of these elements is different. Figure 3.1 compares the elemental composition of the crust of the earth with that typical of a living organism. Obviously, living organisms are not simply a reflection of the chemical composition of their environment. Some elements found abundantly in the

Estimated distribution of elements in the crust of the earth, including land, air, and water

	% by weight
Oxygen (O)	49.5
Silicon (Si)	25.3
Aluminum (Al)	7.5
Iron (Fe)	5.08
Calcium (Ca)	3.39
Sodium (Na)	2.63
Potassium (K)	2.40
Magnesium (Mg)	1.93
	97.69%
Hydrogen (H)	0.87
Titanium (Ti)	0.63
Chlorine (Cl)	0.19
Phosphorus (P)	0.12
	99.50%
Manganese (Mn)	0.090
Carbon (C)	0.080
Sulfur (S)	0.060
Barium (Ba)	0.040
Chromium (Cr)	0.038
Nitrogen (N)	0.030
Fluorine (F)	0.026
Zirconium (Zr)	0.023
Strontium (Sr)	0.020
Nickel (Ni)	0.018
Zinc (Zn)	0.017
Vanadium (V)	0.018
Copper (Cu)	0.010
Total	99.96%

Estimated distribution of elements in living things

	% by weight
O	65
C	18
H	10
N	3
Ca	2
P	1
	99%
K, S, Cl, Na, Mg, Fe	0.9%
Mn, Cu, I (iodine), Co (cobalt), Zn; B (boron), Mo (molybdenum), F, Si, V, Cr, Sn (tin), Se (selenium)	0.1%
Total	100.0%

Fig. 3.1 The distribution of elements in the crust of the earth compared with their distribution in living organisms. The values for any particular organism may vary somewhat from those given here.

Living things accumulate and concentrate a number of elements that are relatively rare in the nonliving environment

nonliving world (e.g. aluminum) appear to play no role in living things. On the other hand, carbon, hydrogen, and nitrogen are found greatly concentrated in living organisms. In fact, these three elements plus oxygen make up 96% of all living material. It looks as though one feature of life is its ability to accumulate and concentrate elements that are relatively rare in the nonliving environment.

Other elements are essential to life, too. Calcium, phosphorus, sodium, potassium, magnesium, chlorine, sulfur, and iron are always found in living things. Added to the previous four, these account for 99.9% of the weight of living organisms. The remaining one-tenth of one percent is made up of a few other elements such as copper, manganese, zinc, cobalt, iodine, vanadium, fluorine, molybdenum, and (in plants only) boron. Because these latter elements are present in such tiny quantities, they are called trace elements. This does not mean that they do not play an important role in life. It is simply that a little goes a long way.

Trace elements

Fig. 3.2 Periodic table of the elements.

KEY

Electrons in 1st shell → 2
Electrons in 2nd shell → 8
Electrons in 3rd shell → 18
Electrons in 4th shell → 32
Electrons in 5th shell → 18
Electrons in 6th shell → 8
Electrons in 7th shell → 2

Radium ← Name
88 Ra ← Atomic number, Symbol
(226) ← Atomic weight

Note:
( ) indicates most stable or best known isotope.

1																		2
Hydrogen **1 H** 1.00797																		Helium **2 He** 4.0026
2-1 Lithium **3 Li** 6.939	2-2 Beryllium **4 Be** 9.0122											2-3 Boron **5 B** 10.811	2-4 Carbon **6 C** 12.0111	2-5 Nitrogen **7 N** 14.0067	2-6 Oxygen **8 O** 15.9994	2-7 Fluorine **9 F** 18.9984	2-8 Neon **10 Ne** 20.183
2-8-1 Sodium **11 Na** 22.9898	2-8-2 Magnesium **12 Mg** 24.312											2-8-3 Aluminum **13 Al** 26.9815	2-8-4 Silicon **14 Si** 28.086	2-8-5 Phosphorus **15 P** 30.9738	2-8-6 Sulfur **16 S** 32.064	2-8-7 Chlorine **17 Cl** 35.453	2-8-8 Argon **18 Ar** 39.948
2-8-8-1 Potassium **19 K** 39.102	2-8-8-2 Calcium **20 Ca** 40.08	2-8-9-2 Scandium **21 Sc** 44.956	2-8-10-2 Titanium **22 Ti** 47.90	2-8-11-2 Vanadium **23 V** 50.942	2-8-13-1 Chromium **24 Cr** 51.996	2-8-13-2 Manganese **25 Mn** 54.938	2-8-14-2 Iron **26 Fe** 55.847	2-8-15-2 Cobalt **27 Co** 58.933	2-8-16-2 Nickel **28 Ni** 58.71	2-8-18-1 Copper **29 Cu** 63.54	2-8-18-2 Zinc **30 Zn** 65.37	2-8-18-3 Gallium **31 Ga** 69.72	2-8-18-4 Germanium **32 Ge** 72.59	2-8-18-5 Arsenic **33 As** 74.922	2-8-18-6 Selenium **34 Se** 78.96	2-8-18-7 Bromine **35 Br** 79.909	2-8-18-8 Krypton **36 Kr** 83.80
2-8-18-8-1 Rubidium **37 Rb** 85.47	2-8-18-8-2 Strontium **38 Sr** 87.62	2-8-18-9-2 Yttrium **39 Y** 88.905	2-8-18-10-2 Zirconium **40 Zr** 91.22	2-8-18-12-1 Niobium **41 Nb** 92.906	2-8-18-13-1 Molybdenum **42 Mo** 95.94	2-8-18-13-2 Technetium **43 Tc** (99)	2-8-18-15-1 Ruthenium **44 Ru** 101.07	2-8-18-16-1 Rhodium **45 Rh** 102.905	2-8-18-18 Palladium **46 Pd** 106.4	2-8-18-18-1 Silver **47 Ag** 107.870	2-8-18-18-2 Cadmium **48 Cd** 112.40	2-8-18-18-3 Indium **49 In** 114.82	2-8-18-18-4 Tin **50 Sn** 118.69	2-8-18-18-5 Antimony **51 Sb** 121.75	2-8-18-18-6 Tellurium **52 Te** 127.60	2-8-18-18-7 Iodine **53 I** 126.904	2-8-18-18-8 Xenon **54 Xe** 131.30
2-8-18-18-8-1 Cesium **55 Cs** 132.905	2-8-18-18-8-2 Barium **56 Ba** 137.34	**57-71** See Lanthanide Series	2-8-18-32-10-2 Hafnium **72 Hf** 178.49	2-8-18-32-11-2 Tantalum **73 Ta** 180.948	2-8-18-32-12-2 Wolfram **74 W** 183.85	2-8-18-32-13-2 Rhenium **75 Re** 186.2	2-8-18-32-14-2 Osmium **76 Os** 190.2	2-8-18-32-15-2 Iridium **77 Ir** 192.2	2-8-18-32-17-1 Platinum **78 Pt** 195.09	2-8-18-32-18-1 Gold **79 Au** 196.967	2-8-18-32-18-2 Mercury **80 Hg** 200.59	2-8-18-32-18-3 Thallium **81 Tl** 204.37	2-8-18-32-18-4 Lead **82 Pb** 207.19	2-8-18-32-18-5 Bismuth **83 Bi** 208.980	2-8-18-32-18-6 Polonium **84 Po** (210)	2-8-18-32-18-7 Astatine **85 At** (210)	2-8-18-32-18-8 Radon **86 Rn** (222)
2-8-18-32-18-8-1 Francium **87 Fr** (223)	2-8-18-32-18-8-2 Radium **88 Ra** (226)	**89-103** See Actinide Series	**104**		Hahnium **105 Ha** (260)												

Lanthanide Series (Rare Earth Elements)	2-8-18-18-9-2 Lanthanum **57 La** 138.91	2-8-18-19-9-2 Cerium **58 Ce** 140.12	2-8-18-21-8-2 Praseodymium **59 Pr** 140.907	2-8-18-22-8-2 Neodymium **60 Nd** 144.24	2-8-18-23-8-2 Promethium **61 Pm** (147)	2-8-18-24-8-2 Samarium **62 Sm** 150.35	2-8-18-25-8-2 Europium **63 Eu** 151.96	2-8-18-25-9-2 Gadolinium **64 Gd** 157.25	2-8-18-26-9-2 Terbium **65 Tb** 158.924	2-8-18-28-8-2 Dysprosium **66 Dy** 162.50	2-8-18-29-8-2 Holmium **67 Ho** 164.930	2-8-18-30-8-2 Erbium **68 Er** 167.26	2-8-18-31-8-2 Thulium **69 Tm** 168.934	2-8-18-32-8-2 Ytterbium **70 Yb** 173.04	2-8-18-32-9-2 Lutetium **71 Lu** 174.97
Actinide Series	2-8-18-32-18-9-2 Actinium **89 Ac** (227)	2-8-18-32-18-10-2 Thorium **90 Th** 232.038	2-8-18-32-20-9-2 Protactinium **91 Pa** (231)	2-8-18-32-21-9-2 Uranium **92 U** 238.04	2-8-18-32-22-9-2 Neptunium **93 Np** (237)	2-8-18-32-23-9-2 Plutonium **94 Pu** (242)	2-8-18-32-24-9-2 Americium **95 Am** (243)	2-8-18-32-25-9-2 Curium **96 Cm** (247)	2-8-18-32-26-9-2 Berkelium **97 Bk** (247)	2-8-18-32-27-9-2 Californium **98 Cf** (251)	2-8-18-32-28-9-2 Einsteinium **99 Es** (254)	2-8-18-32-29-9-2 Fermium **100 Fm** (253)	2-8-18-32-30-9-2 Mendelevium **101 Md** (256)	Nobelium **102 No** (254)	Lawrencium **103 Lw** (257)

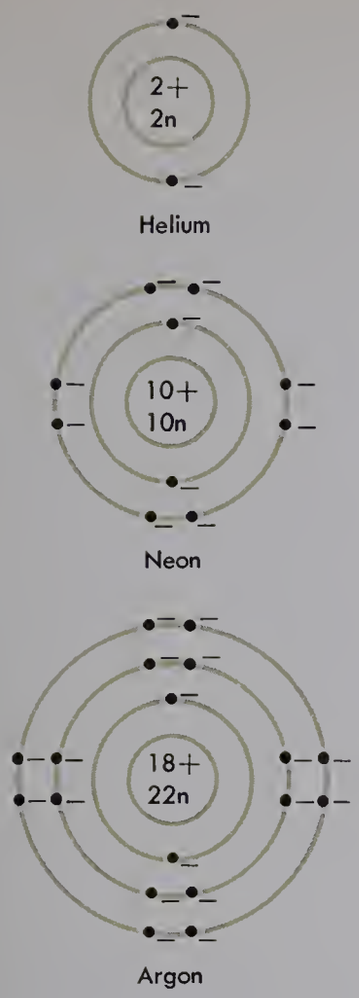


Fig. 3.3 The atomic structure of helium, neon, and argon. In each case there is a full complement of electrons in the outermost shell. Consequently, these elements do not react readily with other elements. They are called inert gases.

3.2 ATOMS

Each of the 105 elements known to man is composed of one particular kind of atom. We can define an atom as the smallest part of an element that can enter into combinations with other elements. (More of this in a moment.) For ease in discussing elements and the atoms of which they are composed, we use symbols, one for each element (Fig. 3.2).

We have said that elements cannot be further decomposed by chemical means. The same is true, of course, of the atoms of which they are composed. Atoms can, however, be decomposed by the use of more violent, physical methods. Years of such work have shown that atoms, too, have a very definite and orderly structure.

The simplest atom, the hydrogen atom, consists of a single positively charged particle, the **proton,** surrounded by a single negatively charged particle, the **electron.** The two charges are equal in magnitude so the atom is electrically neutral. Almost all the weight of the atom is accounted for by the weight of the proton.

The element with the next most complicated atom is the gas helium (He). Its atoms have two protons and also two other particles called **neutrons.** Neutrons are the same weight as protons but do not have any electrical charge. Both the protons and neutrons adhere tightly together to form the dense, positively charged nucleus of the atom. Around this nucleus are two electrons so that, once again, the atom as a whole is neutral.

The structure of all the other atoms follows the same plan. Each of the other 103 kinds, from lithium (No. 3) to hahnium (No. 105), consists of one more proton and one more electron than the atom just before it in the list (Fig. 3.2). Different atoms of a single element may have a different number of neutrons in the nucleus (these variant atoms are called **isotopes**), but this has little or no effect on the chemical properties of the atom.

The electrons surrounding the nucleus of each atom are restricted to certain definite "shells" or energy levels. These shells can be thought of as existing on the surface of a hollow sphere of a definite radius. Furthermore, there are limits to the number of electrons that can be present at any single energy level. At the innermost energy level only two can be present. Thus this level is filled in the helium atom. The next level can hold a maximum of eight electrons. These are arranged in four pairs. The atom of the gas neon has eight electrons in this second shell (Fig. 3.3). The maximum number of electrons which can exist at any given level (n) can be computed from the expression

$$\text{maximum number} = 2n^2.$$

Limits on the number of electrons that can occupy a given energy level

Thus, the third energy level can hold up to 18 electrons (nine pairs). How many electrons can exist at the fourth level?

There is one other important rule that describes the arrangement of electrons in the electrically neutral atom: no more than eight electrons will be found in any energy level when it is the outermost one. For example, the third energy level can hold up to 18 electrons. It cannot hold more than eight, however, unless there are electrons in the fourth shell.

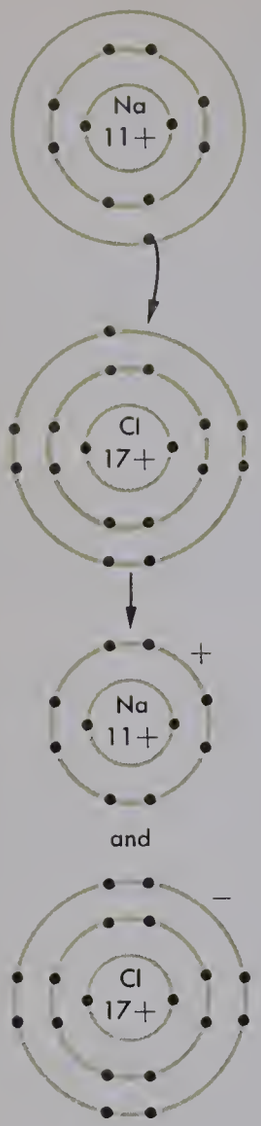


Fig. 3.4 Formation of sodium chloride. In the transfer of an electron from a sodium atom to a chlorine atom, each atom acquires an outer shell of 8 electrons and thus attains stability. Each also acquires an electrical charge. Charged atoms are called ions.

The arrangement of electrons in the atom plays a crucial role in its chemistry. Atoms are most stable when their outer shell contains its full quota of electrons. The so-called inert gases (e.g. neon and argon) have eight electrons in their outer shell.

Other atoms tend to gain or lose electrons until their outer shell also has the stable arrangement of the inert gases. Atoms with one, two, or three electrons in the outer shell usually lose these so that the next inner shell, with a more stable set of electrons, is left exposed. These atoms are called **electropositive.** The elements with atoms of this kind are the metals, such as sodium, magnesium, potassium, and calcium. Atoms with four, five, six, or seven electrons in their outer shell tend to add the necessary number of electrons to complete this shell. These atoms are **electronegative.** The elements with atoms of this kind are the nonmetals, such as carbon, nitrogen, oxygen, phosphorus, sulfur, and chlorine. The tendency for atoms to achieve a stable arrangement of electrons in the outer energy level explains why the various elements combine with one another the way they do. For example, table salt, sodium chloride, is made from equal numbers of sodium and chlorine atoms. The sodium atoms, with one electron in their outer shell, release this electron and thus gain stability. The chlorine atoms, with seven electrons in their outer shell, take on the electron from the sodium atoms and likewise gain stability (Fig. 3.4).

Our emphasis on the stable arrangements of electrons in an atom should not obscure the fact that unstable arrangements can exist. By adding energy to an atom, it is possible to "kick" electrons from a lower to a higher energy level. Such an atom is known as an "excited" atom. It is unstable. If left alone, the electron will soon return to its former energy level. In so doing, it will give back most of the energy that it had absorbed in the first place. When certain minerals are placed under ultraviolet light, they glow in brilliant colors. This is called **fluorescence.** It occurs because the electrons in the atoms of the mineral absorb energy from the ultraviolet light and are raised to a higher energy level. An instant later, however, they return to their former positions, giving up the absorbed energy in the form of visible light. (Fluorescent lamps work on exactly the same principle.) We shall see later on that this ability of electrons to store energy is vitally important to the existence of life itself.

One other property of atoms that we should note is the fact that they have weight. Almost all of this is accounted for by the weight of the protons and neutrons in the nucleus. Hydrogen, with just a single proton in its nucleus, is the lightest atom. Uranium, with 92 protons and 146 neutrons in its nucleus, is the heaviest naturally occurring atom.

Atomic weight

The weight of any single atom is, of course, infinitesimal when calculated in common units such as grams or ounces. To make calculations easier, we express the weights of atoms in terms of a special unit, the **atomic weight unit** or **dalton.** This unit is set arbitrarily as one-twelfth of the weight of a single atom of carbon (with six protons and six neutrons). Such a unit is very convenient because it makes the lightest element, hydrogen, have an atomic weight of 1. (Why?) It also makes

individual protons and neutrons have a weight of 1. Thus the most common type of oxygen atom, which has eight protons and eight neutrons in its nucleus, has an atomic weight of 16. It is designated as ^{16}O. It is only one of three **isotopes** of oxygen found in the atmosphere. The others, however, make up less than 1% of the total number of oxygen atoms present. One of these isotopes has 8 protons and 9 neutrons in its nucleus, and thus its atomic weight is 17. Its symbol is ^{17}O. The other, ^{18}O, has 8 protons and 10 neutrons in its nucleus and thus an atomic weight of 18. Note that all the isotopes of oxygen have just 8 protons in the nucleus.

3.3 CHEMICAL COMPOUNDS

A chemical compound is a substance that can be decomposed into two or more simpler substances. If these simpler substances are also compounds, then they can be further decomposed. Ultimately, compounds can be decomposed into elements. The amounts of the elements in a given compound are not variable but are always present in definite proportions by weight. This is a reflection of the fact that the atoms of the elements in a compound are attached to one another in a precise way. Water is a compound. It is made up of the elements hydrogen and oxygen. The weight of the hydrogen in the compound is one-eighth the weight of the oxygen. This is understandable if two atoms of hydrogen (atomic weight = 1) are attached to each oxygen atom (atomic weight = 16) in the compound.

The properties of a compound are different from those of the elements of which it is composed

One of the most important things to remember about compounds is that their properties, such as color, taste, chemical activity, etc., are usually quite different from the properties of the elements of which they are made. The element sodium is poisonous and reacts explosively with water. The element chlorine was used as a poison gas during World War I. The compound sodium chloride, however, is absolutely vital to life activities. Its properties are quite different from those of either sodium or chlorine. Similarly, the properties of water are not the properties of the hydrogen or oxygen of which it is composed.

3.4 MOLECULES

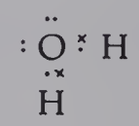
Molecular weight

Most of the compounds of special interest to biologists are made up of units called molecules. Each molecule consists of a precise arrangement of the atoms of the elements in the compound. It is this precise arrangement which accounts for the fixed ratio of elements in the compound. Because the constituent atoms have weight, the molecules have weight. The molecular weight (M.W.) is equal to the sum of the atomic weights of the atoms in the molecule. The molecular weight of water is 18. What is the molecular weight of carbon dioxide, CO_2?

It should be noted that many common gaseous *elements,* e.g. hydrogen, oxygen, nitrogen, and chlorine, are also composed of molecules. In these cases, only two atoms are present in the molecule, and the two atoms are alike. We designate these molecules as H_2, O_2, N_2, and Cl_2 respectively.

A molecule is a particle containing two or more atoms linked by covalent bonds

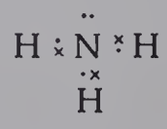
As we have seen, water is made up of molecules consisting of one oxygen atom to which are attached two hydrogen atoms. These atoms are held together by their tendency to achieve the most stable arrangement of electrons in their outer shells. Oxygen, with six electrons in its second shell, lacks two electrons to make a stable set of eight. Hydrogen, with one electron, needs a second to reach the stable arrangement of two electrons in its shell. By sharing electrons between them, each atom in a molecule of water gains a stable arrangement. Each hydrogen atom contributes an electron to the oxygen but, at the same time, electrons of the oxygen atom contribute to the formation of a pair for each of the hydrogen atoms. We can illustrate this by representing the nucleus (and all electron shells except the outer one) of the atoms by the symbol of the element and indicating the electrons in the outermost shell by dots or small crosses.



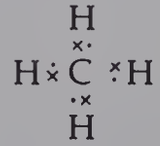
Note that the oxygen atom now is surrounded by eight electrons and each hydrogen atom is surrounded by two. The process of sharing electrons causes the atoms to be linked or bonded together. This kind of bond is known as a **covalent bond.** It occurs between atoms that do not differ greatly in their electronegativity. Therefore, neither atom is able to gain complete control over the bonding electrons. There is not the *transfer* of electrons depicted in Fig. 3.4 but a *sharing* of electrons.

The number of bonds formed by an atom is its valence

The number of electrons that a given atom has available to share in the making of covalent bonds sets a definite limit on its ability to combine with other atoms. Hydrogen, with just one electron to share, can only combine with one other atom. It is said to have a combining power, or **valence,** of one. Oxygen, needing to pair two of its electrons with two from elsewhere in order to achieve a complete set of eight, has a valence of 2. Nitrogen, with five electrons in its outer shell, needs three more electrons to form eight. Hence, it will share three of its electrons with three electrons from elsewhere. It has a valence of 3. The compound of nitrogen and hydrogen consists of one atom of nitrogen and three atoms of hydrogen.



It is the substance ammonia. Carbon, needing four electrons to share with its own four electrons has a valence of 4. Its compound with hydrogen can be expressed as



This substance is a gas, methane, frequently produced in marshes.

Carbon atoms with a valence of 4 and oxygen atoms with a valence of 2 can also unite. In this case, two oxygen atoms unite with one carbon atom to form a molecule of the gas carbon dioxide, CO_2. The electron-dot representation of this molecule is interesting because it shows that not one, but two, pairs of electrons are shared between the respective atoms.



Double bonds

This arrangement is known as a **double** covalent bond. Note that it still provides for a complete set of eight electrons around each atom in the molecule.

3.5 FORMULAS

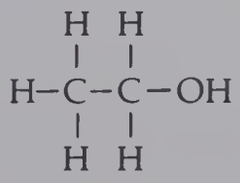
The electron-dot representations of the structures of molecules are a kind of formula. While they tell us a great deal about the arrangement of atoms in the molecule, they are a little complicated for regular use, particularly in considering large, complex molecules. Consequently, chemists have devised other, shorthand expressions for indicating the makeup of a compound.

Molecular formulas list the number of each kind of atom in the molecule

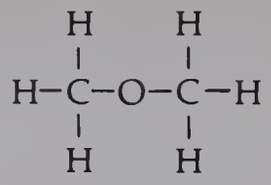
One of these is the molecular formula. This formula simply lists all the kinds of atoms found in the molecule and, after each atom, includes a subscript giving the number of times (over one) that the atom appears in the molecule. The molecular formula for water is H_2O. The molecular formula for a molecule of the milk protein beta-lactoglobulin is approximately $C_{1864}H_{3012}O_{576}N_{468}S_{21}$. Note that the molecular formula simply gives the number of each of the various atoms that are found in the molecule. It does not tell us anything about the way in which these atoms are bonded together. In the case of beta-lactoglobulin, in fact, the arrangement of the atoms in the molecule is not yet known.

Structural formulas show how each atom in the molecule is bonded to the others

For many molecules we do know the actual physical arrangements of the atoms in the molecule. This is very important information, too, because the properties of a compound depend greatly on the actual way the atoms are linked together. For example, the common beverage alcohol, ethanol, has the formula C_2H_6O. However, there is another compound, an ether (related to the ether commonly used as an anesthetic), that also has the formula C_2H_6O. The difference in properties of these two compounds can be explained by the difference in the way the atoms are assembled in the molecule. We can show this difference by drawing a **structural formula** of each. In a structural formula we use a dash to represent each of the covalent bonds (which we showed above by pairs of dots and crosses). Using this system, we represent the ethanol molecule thus:



On the other hand, the atoms of the ether molecule are attached to each other like this:



Isomers have the same molecular formula but a different structural formula

Note that each molecule has exactly the same number of atoms in it. Note also that in each case all of the valences are satisfied. Each of the two carbon atoms forms four bonds, the oxygen atom has two bonds, and each of the hydrogens forms only one bond. Two molecules, such as these, that have the same molecular formula but different structural formulas are called **isomers.** Isomers are found frequently in compounds containing carbon.

Even structural formulas are a little complex to use all the time. Consequently, chemists have devised condensed versions of them which still impart the essential information. Using condensed structural formulas, we can represent ethanol as CH_3CH_2OH or even C_2H_5OH. The ether is represented as CH_3OCH_3. Note that these condensed structural formulas have the great convenience of being able to be typed or printed in a single line. Note also that the distinguishing feature of each molecule, the "active group," is clearly shown. All alcohols have the –OH group. All ethers have the –O– arrangement.

Determining molecular structure

You may well ask how chemists are able to determine the structural formula of a molecule. The first step is to determine its molecular formula. This is done by carefully decomposing a weighed amount of the compound into its constituent elements (or simple, inorganic molecules of these elements such as CO_2 and H_2O). From the weights of the breakdown products, it is possible to compute the relative numbers of the different atoms in the original molecule. A knowledge of the molecular weight gives the actual numbers and thus the molecular formula. (See question 10.)

There is no single next step in the procedure for determining the structural formula. The process depends upon the vast amount of knowledge about chemical properties of substances and "active groups" that has been accumulated by chemists for well over 100 years. Taking our earlier example, chemists know that the rules of valence will permit two carbon atoms, six hydrogen atoms, and one oxygen atom to be combined in just two ways. Through the years it has been found that alcohols react with other substances in certain ways, ethers in different ways. By finding out whether the chemical activity of our unknown compound is characteristic of the –OH group or the –O– group, we can hope to establish the true structural formula. The ultimate test comes, however, when we synthesize a compound, using another body of principles and rules, with the structural formula we have tentatively assigned to our "unknown." If the synthesized compound has the same physical and chemical properties as our "unknown," we can be quite confident that we do indeed understand the way the molecule is put together.

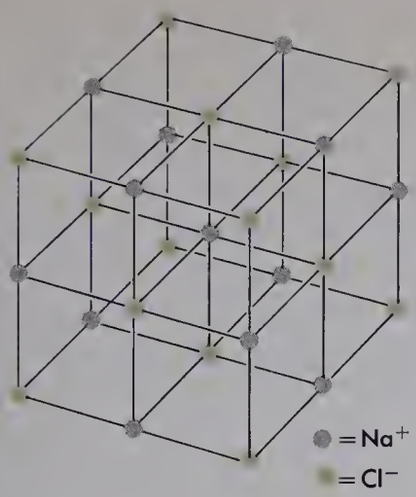


Fig. 3.5 The lattice structure of a crystal of NaCl. The orderly stacking of Na^+ and Cl^- ions produces a crystal in the shape of a cube.

3.6 IONIC COMPOUNDS

Not all compounds are made of molecules. When atoms combine to form many compounds, the affinity of one of the atoms for electrons is so powerful that it is able to remove outer electrons completely from the other atom or atoms. Table salt is a good example of this. Chlorine, with its one missing electron, is so strongly **electronegative** that it completely pulls away the one outer electron of the sodium atom (Fig. 3.4). Sodium, like all metals, does not have a great affinity for its outer electron so it gives its electron up readily. Having lost an electron, however, it now possesses a single positive charge (11 protons, but only 10 electrons). Similarly, the chlorine atom now has a single negative charge because of its additional electron (17 protons, 18 electrons). These charged atoms are called **ions.** The mutual attraction of opposite electrical charges holds the ions together by **ionic** bonds. The ions are not held together in pairs but are stacked in three-dimensional arrays (Fig. 3.5). Each sodium ion is held to six chloride ions (above, below, front, back, left, and right) while each chloride ion is, in turn, held by six sodium ions. The result of this stacking of ions is a crystal of table salt. At no place can one single out a pair of ions and refer to it as a molecule.

Formulas of ionic compounds

Since compounds that are ionically bonded contain no molecules, can we write formulas for them? Certainly, but in this case we are restricted to a formula that simply shows the ratios of the various positive and negative ions in the crystal. The formula NaCl shows that these two ions are present in a one-to-one ratio. Magnesium, with a valence of 2, forms crystals with two chloride ions for every one magnesium ion. The formula for this compound is $MgCl_2$. What would be the formula for aluminum chloride?

Because ionic compounds are not made of molecules, we cannot properly speak of molecular weight. We can easily arrive at the equivalent value, however, by adding up the atomic weights of the minimum ratio of ions in the crystal. We call this value the **formula weight.** The formula weight of sodium chloride is 58.5 (Na = 23, Cl = 35.5).

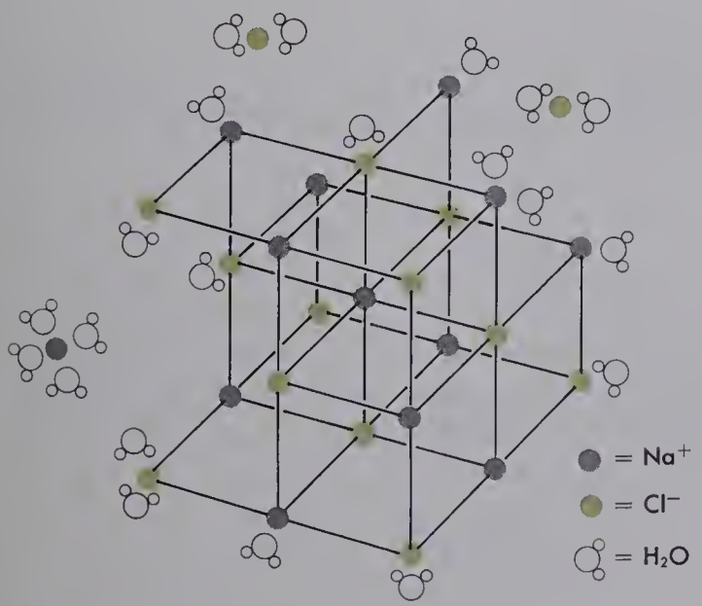


Fig. 3.6 Dissolving the crystal lattice of NaCl. Although the water molecule as a whole is electrically neutral, the uneven distribution of its electrons causes it to be somewhat polar, i.e. negatively-charged around the oxygen atom and positively-charged around the two hydrogens. Because of this polarity, water is an excellent solvent. The Cl^- ions are attracted to the positively-charged region of water molecules; the Na^+ ions to the negatively-charged region. As a result, the ions become dissociated and the crystal dissolves.

Ionic compounds are widespread in nature. Although we naturally associate them with the nonliving world of rocks and minerals, many ionic compounds are essential to life. Salts of sodium, potassium, calcium, chlorine, and other elements are found dissolved in the water of blood, cell fluid, etc. In this condition, the positive and negative ions become separated or **dissociated** from each other. (Fig. 3.6). The essential chemical properties of the substance are not changed in the process, however. Sodium ions (Na^+) and chloride ions (Cl^-) dissolved in water still retain the properties of table salt. They do not regain the poisonous properties of the neutral atoms from which these ions were made.

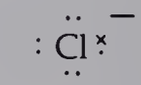
3.7 ACIDS

Molecules can also form ions

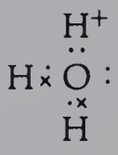
Even the molecules of covalently bonded compounds may dissociate into ions when they dissolve in water. Many covalent compounds containing hydrogen atoms are able to release one or more of them to nearby water molecules. For example, the gas hydrogen chloride is made up of molecules containing the two atoms held together by a shared pair of electrons, a covalent bond:



When this gas dissolves in water, the nucleus of the hydrogen atom (a proton) is attracted to one of the unshared electron pairs of a nearby water molecule. This leaves a negatively charged chloride ion

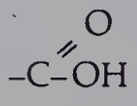


and produces a positively charged **hydronium** ion, H_3O^+:



When a substance gives up a proton, it is acting as an acid

The resulting solution is hydrochloric acid. It has a sour taste and other properties associated with acids. Any organic compound that possesses the **carboxyl** group



will also dissociate into ions when placed in water. One of these will be a proton (H^+) which will immediately combine with a water molecule to form the hydronium ion (H_3O^+). Acetic acid has one carboxyl group and ionizes to form an acetate ion (CH_3COO^-) and a proton.

$$CH_3COOH + H_2O \longrightarrow CH_3COO^- + H_3O^+$$

Any compound that liberates protons in water solution is an acid.

3.8 CHEMICAL CHANGES

Chemical changes are changes in which new substances with new properties are formed. When hot sodium is surrounded by chlorine gas, the sodium bursts into flame. Table salt, sodium chloride, results. This is an example of a chemical change. The burning of coal is another example. Can you think of others?

All chemical changes involve a rearrangement of atoms (or ions). We can express these changes by means of an equation. To write a chemical equation we must first write the formulas of all the substances used in the chemical change. These are the **reactants.** Then we draw an arrow and write the formulas of all the substances produced by the chemical change, the **products.** When ethanol (ethyl alcohol) is exposed to air and the action of vinegar bacteria, it is changed chemically into vinegar (acetic acid) and water. We express this change by the equation

$$C_2H_5OH + O_2 \longrightarrow CH_3COOH + H_2O$$

Writing chemical equations

Note carefully that every atom present on the left side of the equation is accounted for on the right. If it is not, then the equation does not give a true picture of the chemical change. This is simply another way of saying that matter can neither be created nor destroyed. (We may neglect the special case of nuclear changes that occur in atom bombs, etc.)

In many chemical changes, the substances do not react in simple one-to-one ratios as they do in the production of acetic acid. When magneisum is burned in air, for example, two atoms of magnesium react with just a single molecule of oxygen. Two parts of the white powder magnesium oxide are produced. We express these relationships by placing the prefix 2 before the symbol for magnesium and the formula for magnesium oxide:

$$2Mg + O_2 \longrightarrow 2MgO$$

You can easily carry out this chemical change yourself. Grasp a small piece of magnesium ribbon in a spring-type clothespin and hold the tip of the ribbon in a candle or bunsen burner flame. The heat of the flame will first soften the ribbon. Then the ribbon will begin to burn. In so doing, it gives off a good deal of heat and a brilliant light. (Do not stare at the light.) When the burning is completed, a white powder, magnesium oxide, remains.

An equation reveals the relative weights of the reactants and products

An equation can tell us even more than what rearrangement of atoms has taken place. It can also tell us what the relative weights of the reactants and products of the chemical change are. From the equation given above, you might think that 1 gram (gm) of ethanol and 1 gm of oxygen would produce 1 gm of acetic acid and 1 gm of water. Such is not the case, however. The equation tells us that one molecule of ethanol reacts with one molecule of oxygen. It implies that likewise a billion molecules of one will react with exactly a billion molecules of the other. Thus, the 1:1 ratio expresses the number of each type of molecule in the reaction. You can quickly see by computing the molecular weights of these two molecules

that the ethanol molecule is somewhat heavier (M.W. = 46) than the oxygen molecule (M.W. = 32). If we want a given quantity of ethanol to react completely with oxygen, we must supply one molecule of oxygen for every molecule of ethanol. Counting molecules is not practical. Weighing substances, on the other hand, is a very efficient technique. To make sure that equal numbers of ethanol and oxygen molecules react, we must bring about a reaction between weights of these two substances which are in the ratio of 46:32.

Another example may help make this point clear. As a biology student, you might be interested in comparing the effects of sodium chloride and potassium chloride solutions on the rate of beating of an isolated frog heart. At first you might plan to prepare one-percent solutions (1 gm of the salt in 99 gm of water) of each substance. This would be a poor technique, however. The formula weights of these two salts are 58.5 and 74.5 respectively. A one-percent solution of sodium chloride would thus contain almost half again as many ions as a one-percent solution of the potassium chloride. (Why?) In such a case, you could not properly relate the response of the frog heart to the kind of metal ion applied.

The mole

A much better technique is to make up the salt solutions so that equal volumes of each of the two solutions contain equal numbers of the ions. To do this, we simply weigh out 58.5 parts of NaCl to every 74.5 parts of KCl. Then these two quantities must each be added to enough water to make the two resulting solutions of exactly equal volume. Once this is done, you know that, drop for drop, the two solutions have exactly the same concentration in terms of numbers of ions present.

In trying to weigh out two substances in the ratio of 58.5 to 74.5, what could be more simple than to weigh out 58.5 gm of one and 74.5 gm of the other? In so doing, you would have weighed out a quantity of each substance called a **mole.** If you then add enough water to each substance to make exactly one liter of solution, you will have made a 1-molar (1-*M*) solution of each salt. [A specially graduated flask is a useful device in which to do this. After the substance is added to the flask, enough water is added to bring the volume up to the engraved line on the neck of the flask. The volume of solution is then exactly one liter (Fig. 3.7).] A 1-*M* solution of these salts would undoubtedly be too strong for the experiment on the frog heart. It would probably be better to make up a liter of each solution containing 5.85 and 7.45 gm respectively. Such solutions would be designated one-tenth molar (0.1*M*) solutions. Drop for drop, these two solutions would also contain exactly the same number of ions because they are of the same molarity.



Fig. 3.7 A volumetric flask. When filled to the etched line, it contains exactly one liter. Such a flask is used to prepare solutions of precise molarity.

Looking back at our earlier example, we are now in a position to estimate how much acetic acid and how much water would be produced as a result of reacting one mole of ethanol (46 gm) with one mole of oxygen (32 gm). One mole of each substance would be produced, namely 60 gm of CH_3COOH and 18 gm of water. Note, also, that the total weight of the products (60 gm + 18 gm = 78 gm) is exactly equal to the total weight of the reactants (46 gm + 32 gm = 78 gm). This, once again, illustrates the

principle that during chemical changes, matter is neither created nor destroyed. (In practice, when organic compounds react, one seldom gets a mole of each product for each mole of reactant, i.e. 100% efficiency. This is not because any matter is lost. It is simply because other reactions, "side" reactions, go on simultaneously and produce unwanted products.)

3.9 TYPES OF CHEMICAL CHANGE

There are several types of chemical change. Two of these are of special importance to the beginning biology student. These are ion-exchange reactions and oxidation-reduction (redox) reactions.

1. Ion-Exchange Reactions

When hydrogen chloride gas (HCl) is dissolved in water, a solution of hydrochloric acid is produced.

$$HCl + H_2O \longrightarrow H_3O^+ + Cl^-$$

As explained earlier, this reaction simply involves the transfer of an ion, a proton (H^+), from one molecule to another. There has been no change in the arrangement of the electrons. The HCl molecule donated the proton and, like all proton donors, is called an acid. The water molecule, which accepts the proton, acts as a **base.** It is not a very strong base, though, because it will readily give its proton up to other proton acceptors. One of the most powerful of these is the hydroxyl ion. This ion is produced when substances called hydroxides (e.g. NaOH) are dissolved in water. The OH^- ion has a greater affinity for protons than does the water molecule. Consequently, the proton shifts from the hydronium ion, now the acid, to the hydroxyl ion, the base.

When a substance accepts a proton, it is acting as a base

$$H_3O^+ + OH^- \longrightarrow 2H_2O$$

Acid-base reactions

If one adds equal volumes of an acid solution and a basic solution (of the same concentration of reacting ions) together, they will react completely in this way. This process is called **neutralization** because the resulting solution has neither the properties of an acid nor the properties of a base. In fact, the solution produced by this particular neutralization is simply salt water.

$$H_3O^+ + Cl^- + Na^+ + OH^- \longrightarrow 2H_2O + Na^+ + Cl^-$$

The OH^- ion is a very powerful proton acceptor and thus a very strong base. It is never found in high concentrations in living organisms. Probably the most important base in living tissue is the bicarbonate ion, HCO_3^-. This base readily neutralizes excess acid that may be formed by cell metabolism. Perhaps you know of people who take sodium bicarbonate to neutralize the hydrochloric acid in their stomachs in an attempt to alleviate "acid indigestion."

$$H_3O^+ + Cl^- + Na^+ + HCO_3^- \longrightarrow H_2O + H_2CO_3 + Na^+ + Cl^-$$

In summary, when any molecule or ion gives up a proton, it is acting as an acid. When any molecule or ion takes on a proton, it is acting as a base. Any acid and any base will neutralize each other if each solution contains the same number of ions.

2. Oxidation-Reduction Reactions

As one atom is oxidized, another must be reduced

Oxidation-reduction reactions differ from ion-exchange reactions in that a transfer of *electrons* is always involved. You remember that when sodium burns in chlorine (Fig. 3.4) each sodium atom loses an electron to a chlorine atom. The atom that loses the electron (sodium) is said to have been **oxidized.** The atom that gains the electron (chlorine) is said to have been **reduced.** The two processes are completely linked. Whenever any substance gives up electrons, it is oxidized. The substance to which it gives its electrons is called the oxidizing agent. Note, however, that any substance, in the process of acting as an oxidizing agent, is itself reduced. Because oxidations always go hand-in-hand with reductions, we refer to these reactions as redox reactions.

Oxygen is only one of many oxidizing agents

You may wonder why the reaction between sodium and chlorine is called an oxidation when oxygen plays no part in it. The answer is simply a matter of history. Oxygen has a great affinity for electrons and is thus one of the best and most common oxidizing agents known. Because of its effectiveness in this role, it has supplied the name for all reactions of this type.

The burning of magnesium is an example of an oxidation that actually does involve oxygen. The outer two electrons on the magnesium atom (an electropositive element—see Section 3.6) are taken on by an atom of oxygen (a strongly electronegative element), thus completing its outer shell of eight electrons. All strongly electronegative elements like oxygen and chlorine are good oxidizing agents. They are very effective at removing electrons from other substances. Conversely, electropositive elements like sodium and magnesium are easily oxidized. They give up their outer electrons easily. They are good reducing agents.

Electropositive elements are good reducing agents

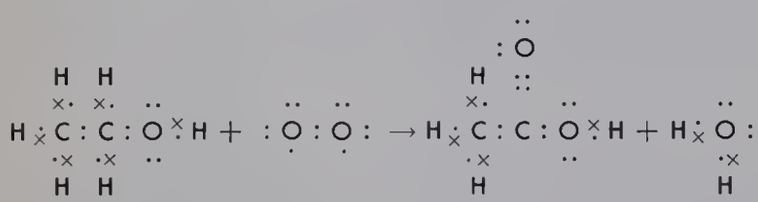


Fig. 3.8 The oxidation of ethanol. Oxidation has occurred both by the addition of an oxygen atom and by the removal of hydrogen atoms. In each case, electrons are moved away from carbon atoms.

Some biological oxidations involve the addition of oxygen to the molecule being oxidized. In the reaction between ethanol and O_2, one atom of oxygen joins the molecule. Because the difference in electronegativity of carbon and oxygen atoms is not as great as, say, that between oxygen and magnesium, the added oxygen atom does not remove electrons sufficiently far to form ions. It nevertheless does attract two electrons away from the carbon atom and much closer to itself (Fig. 3.8).

The removal of hydrogen atoms from a substance is an oxidation

The most common kind of biological oxidation is accomplished by the removal of hydrogen atoms from a substance. The oxidation of ethanol also exhibits this kind of oxidation. Two hydrogen atoms, each with its single electron, are removed by the second oxygen atom. These unite to form a molecule of water.

Although all oxidations go hand-in-hand with reductions, we often emphasize the oxidation part of a redox reaction when the electrons are moving from a weakly electronegative atom to a strongly electronegative one. This is the case with the reaction of magnesium and oxygen (or sodium and chlorine). Such redox reactions are characterized by the liberation of copious amounts of energy and by the formation of very tight bonds. We heat our homes, run our automobiles, and, in fact, extract energy from all our fuels by this sort of redox reaction. The burning of coal can be represented simply by the equation $C + O_2 \longrightarrow CO_2 +$ energy.

The transfer of electrons to a more electronegative atom releases energy

It is also possible to move electrons back from strongly electronegative atoms to weakly electronegative ones. In this case, however, energy must be put into the reaction and we emphasize the reduction aspect of the process. If, for example, we pass a direct current through water, we can break it back down into hydrogen and oxygen. In this case, the oxygen atoms are oxidized (they lose electrons) and the hydrogen atoms reduced. We have, in fact, broken tight bonds (H—O), the formation of which liberated a great deal of energy, and formed weaker bonds (H—H, O—O). Thus weak bonds can serve as a means of storing energy. This energy is chemical, potential energy. As soon as we permit the electrons which are involved to return to more strongly electronegative atoms, the energy is liberated.

Weak bonds store energy

All life depends on redox reactions for trapping and using energy

We shall see in Chapters 8 and 9 that life continues to exist on this earth because it is able to exploit redox reactions. It uses them to store the sun's energy and then to liberate the resulting chemical energy as needed to run all the activities characteristic of living things. In photosynthesis, the energy of the sun is used to move electrons (associated with protons, thus H atoms) from strongly electronegative atoms (O) to weakly electronegative ones (C). In this process strong bonds (H—O, C—O) are broken and weaker bonds are formed (C—H, and especially, O—O). The reverse of this process is cellular respiration. In this case, the electrons return to their original partners and the energy that had been stored is liberated and used to run the organism. Thus it is the energy differences among chemical bonds that makes possible life's storage battery for storing the sun's energy and liberating it as needed to maintain the living process.

Reductions in living organisms are often accomplished by the addition of hydrogen atoms (each with its electron) to a substance. They may also be accomplished simply by the transfer of electrons alone. Some of the metallic elements found in living things have variable valences and play an important part in this. Iron atoms may be quite easily oxidized by the removal of the two electrons in the outer shell.

$$Fe - 2e^- \longrightarrow Fe^{++}$$

A third electron, this one from the shell beneath, can also be removed by oxidation.

$$Fe^{++} - 1e^- \longrightarrow Fe^{+++}$$

Elements with variable valence can participate in redox reactions

The Fe^{+++} ion is a fairly good oxidizing agent itself, however. It can remove an electron from some other substance (which is thus oxidized) and become the Fe^{++} ion once again. Then it can turn right around and act as a mild reducing agent, donating its recently acquired electron to still another substance. This ability of iron to pick up and pass on electrons depends upon its variable valence. Iron and certain other metallic elements play a major role in the redox reactions carried out in living things.

3.10 RATE OF CHEMICAL REACTION

Several factors affect the speed at which chemical changes take place. One of the most important of these is simply the **nature of the reacting materials.** You can well imagine that a vigorous, energy-releasing oxidation, with electrons passing from electropositive to electronegative atoms (as in the burning magnesium ribbon), proceeds much more rapidly than the reverse.

Temperature also affects the rate of chemical reaction. A great deal of the energy liberated by the burning magnesium ribbon is in the form of heat. This heat energizes still-unoxidized magnesium atoms so that they combine even more quickly with oxygen atoms. As a general rule, the speed at which chemical reactions take place doubles with every 10° C rise in temperature. This is true not only for reactions in the test tube but also, within limits, for reactions in living things.

The **concentration** of reacting substances is a third factor in regulating the speed at which chemical changes occur. If the reacting molecules are far apart, their opportunities for colliding and exchanging atoms and electrons are limited. On the other hand, if they are packed closely together, there is a much greater probability of their colliding and of a resulting chemical change.

Atoms cannot interact unless they collide

The reaction $2Mg + O_2 \longrightarrow 2MgO$ involves materials that are brought together whenever a piece of magnesium ribbon is exposed to the air. Why doesn't the reaction proceed spontaneously? Why is it necessary to add energy (heat) to the magnesium ribbon when the reaction between magnesium and oxygen yields such abundant amounts of energy? The answer lies in the need to activate the magnesium atoms. Magnesium atoms will not transfer their electrons to oxygen atoms unless they are activated by the input of energy. At ordinary temperatures, very few atoms become sufficiently active to combine with oxygen. Those that do, however, liberate far more energy in the course of reacting than it took to activate them (Fig. 3.9). Nevertheless, because of the small numbers involved, the resulting heat is liberated so slowly that it does not warm up the metal. Consequently the oxidation of magnesium proceeds very slowly. (The slow oxidation of metals is called corrosion or, in the case of iron, rusting.)

Activation energy

At higher temperatures, a sufficiently large number of magnesium atoms become activated and react with oxygen so that the resulting heat raises the temperature of the metal still further. This activates still other atoms and the reaction becomes self-sustaining. Heat is the most common way in which we supply the energy of activation for chemical reactions. The sparkplug on a gasoline motor and the blasting cap in a stick of dynamite are two examples. Can you think of others?

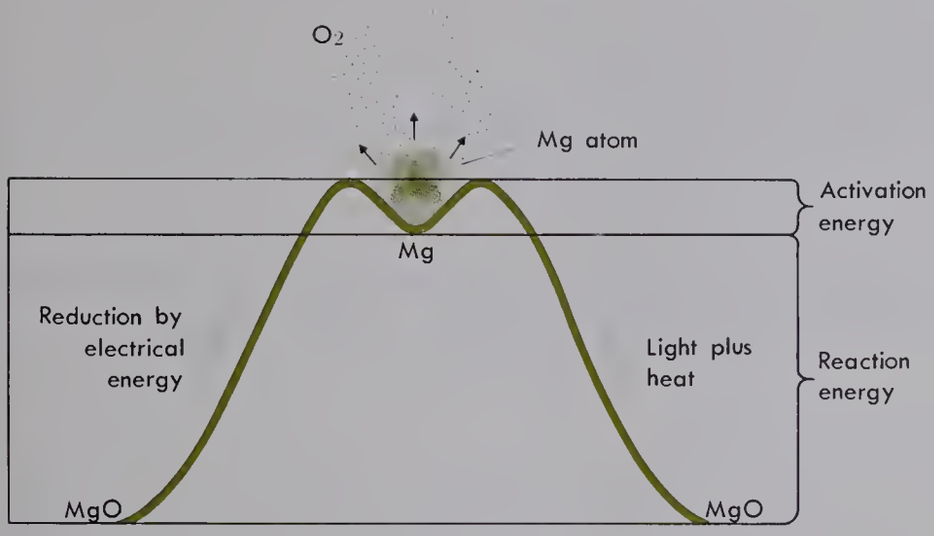


Fig. 3.9 A model showing the energy changes in a redox reaction.

3.11 CATALYSIS

Catalysts reduce the energy of activation

The use of heat to supply activation energy is not too suitable for use by living things. High temperatures are quite destructive to life, as we all know. Fortunately, there is a way around the problem. This is to reduce the energy of activation needed by the use of a suitable **catalyst.** A catalyst is a chemical substance that speeds up the rate at which a given chemical change takes place without becoming permanently altered in the process. Many catalysts accomplish this by temporarily uniting with one of the reacting substances and lowering the amount of activation energy needed. When the reaction is completed, the catalyst is released unchanged and ready for re-use. You can demonstrate catalysis easily with a small quantity of a 3% solution of hydrogen peroxide (H_2O_2)—available in any drug store—and a pinch of the black compound manganese dioxide (MnO_2). At room temperature and atmospheric pressure, and especially when exposed to light, hydrogen peroxide breaks down to form water and oxygen ($2H_2O_2 \longrightarrow 2H_2O + O_2$). (This is why the manufacturer puts the solution up in a brown glass bottle and recommends that it be kept in a cool place with the cap screwed on tightly.) When the solution is poured into a glass, a few bubbles of O_2 soon form. Adding a pinch of MnO_2 greatly increases the speed of the reaction, however. A froth of O_2 bubbles forms immediately (Fig. 3.10). Thus catalysis takes its place as one of the factors that speed up the rate of chemical reactions.

Enzymes are the catalysts of living matter

Living cells have catalysts, too, and thus are able to carry out their chemical activities quickly but at safe temperatures. These catalysts are called **enzymes.** It is thought that every chemical reaction occurring in

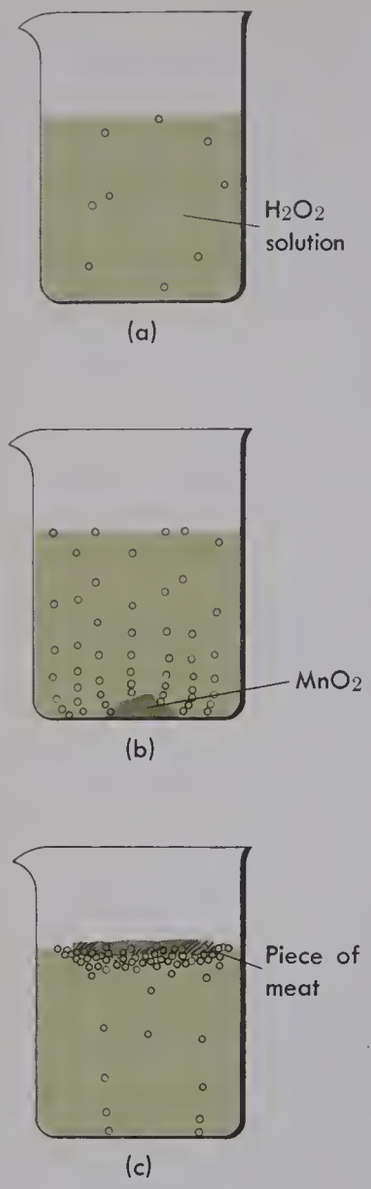


Fig. 3.10 Catalysis. (a) A solution of H_2O_2 spontaneously decomposes, releasing a few bubbles of oxygen. (b) The action is greatly speeded by the addition of the inorganic catalyst manganese dioxide. (c) Cells, in a piece of meat in this case, contain an enzyme, catalase, that likewise hastens the action.

living things (there may be thousands of them) requires the presence of a specific enzyme. Most cells even have an enzyme, called *catalase,* that decomposes H_2O_2 just as MnO_2 does. It is responsible for the frothing which occurs when hydrogen peroxide is poured into an open wound. You can, however, demonstrate the action of the enzyme under more pleasant circumstances by adding a piece of fresh meat to a container of H_2O_2. The meat will quickly float to the top, buoyed up by the bubbles of O_2 that form on its surface.

3.12 REVERSIBLE REACTIONS

In Section 3.9, you learned that the hydronium ion and the bicarbonate ion react to form water and carbonic acid according to the equation:

$$H_3O^+ + HCO_3^- \longrightarrow H_2O + H_2CO_3$$

The carbonic acid (H_2CO_3) that is formed is unstable and decomposes into a molecule of carbon dioxide and a molecule of water:

$$H_2CO_3 \longrightarrow H_2O + CO_2$$

The complete reaction is thus

$$H_3O^+ + HCO_3^- \longrightarrow H_2O + H_2CO_3 \downarrow H_2O + CO_2$$

In a test tube, the carbon dioxide is released into the air, and all the hydronium ions are eventually neutralized. In a closed container, the situation is somewhat different. The carbon dioxide that is produced remains in contact with the water. As the reaction continues, the concentration of CO_2 increases steadily. Soon carbon dioxide molecules are colliding with water molecules and *back-reacting* to form H_2CO_3. Some of this additional H_2CO_3 dissociates in water to form H_3O^+ and HCO_3^-. Reactions in which the products can back-react to form the reactants again are called **reversible.** We use double arrows to indicate the reversible nature of the reaction.

$$H_3O^+ + HCO_3^- \rightleftharpoons H_2O + H_2CO_3 \rightleftharpoons H_2O + CO_2$$

In a closed system, the forward and reverse reactions eventually reach **equilibrium.** At this time, the rate of the forward reactions exactly equals the rate of the reverse reactions. This does not necessarily mean that the concentrations of products and reactants are equal. At equilibrium in the second reaction of our example, in fact, the concentration of CO_2 and H_2O may be some 1000 times greater than that of H_2CO_3. For every molecule of CO_2 that reacts with H_2O, however, a molecule of H_2CO_3 decomposes into H_2O and CO_2. Similarly, at equilibrium, for every hydronium ion that reacts with a bicarbonate ion, a molecule of H_2CO_3 dissociates into its ions.

Chemical equilibrium

Equilibria can be disturbed in several ways. Changing **temperature** usually favors one reaction over the other, and thus brings about a shift

Factors that disturb chemical equilibrium

in the equilibrium. **Adding** fresh **reactants** to the system favors the reaction that consumes the reactants. In our example (see the previous equation) addition of fresh acid displaces the equilibrium to the right, favoring the formation of increased amounts of carbon dioxide. **Removing a product** accomplishes the same thing. As we noted above, in an open test tube the carbon dioxide leaves the reaction mixture and no back reaction is possible. Under these conditions, the forward reaction (neutralization) is said to go to completion.

What about catalysts? Although catalysts have dramatic effects on the rates at which reactions occur, they do not displace reversible reactions in either direction. This is because both the forward and back reactions are speeded up to the same extent. Catalysts thus enable equilibrium to be reached more quickly but do not favor one reaction over its reverse.

The majority of the chemical reactions that occur in living organisms are reversible. Which direction is favored depends upon changes in the factors mentioned above. Proper functioning in the organism depends, in turn, upon the reaction's proceeding in a particular direction at a particular time.

3.13 MIXTURES

The components of a mixture retain their original chemical properties

A mixture is a material composed of two or more pure substances each of which retains its own characteristic properties. When sand is stirred into water, a mixture results. The properties of the sand and the water are unchanged in the process. (You remember that compounds have properties quite different from the properties of their constituents.) Another way in which mixtures differ from compounds is that their composition can be varied. You can mix varying amounts of sand and water together. Magnesium oxide, on the other hand, always contains magnesium atoms and oxygen atoms in the ratio of 24:16 parts by weight.

A mixture like that of sand and water is called a **suspension.** After a period of time, the sand particles (crystals of SiO_2) settle to the bottom of the container under the influence of gravity. Quite a different sort of

Solutions are mixtures

mixture is a **solution.** In a solution, individual molecules or ions of a substance (the **solute**) become suspended in a liquid medium (the **solvent**). These molecules or ions are so small (less than 1 nm) that they never settle out under the influence of gravity. Their own motion counteracts the gravitational force acting on them. When a salt (NaCl, KCl, etc.) or sugar is added to water, a solution results. The mixture is homogeneous, that is, even under the microscope it is perfectly uniform in appearance. While it is easy to separate sand and water by filtering through a cone of paper (Fig. 3.11), solutions cannot be separated in this way. The sizes of the solute molecules and ions in a solution are approximately the same as the size of the solvent molecules and pass through the pores of the filter paper just as easily. Although other liquids can act as solvents, water is the major solvent in living things.

The student of biology should also be familiar with a third kind of mixture, the **colloid.** Colloids are suspensions of particles that are larger

than those in true solutions but still too small to settle out under the influence of gravity. These particles may consist of single, large molecules (1 – 100 nm) or solid clumps of smaller molecules. They are large enough to be seen under very high magnifications. They cannot be filtered out by ordinary filter paper because the pores in the paper are too large to stop their passage. Cellophane, however, has pores sufficiently small to separate colloidal particles from smaller molecules (Fig. 3.12). Egg white is a familiar colloid.

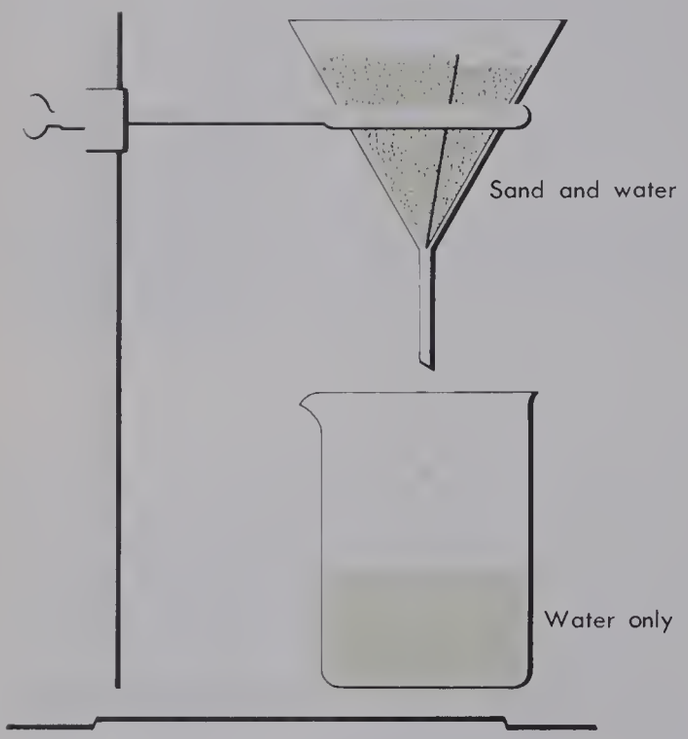


Fig. 3.11 Filtration. Any particles, such as sand, too large to pass through the pores in the paper can be separated in this way from smaller molecules such as those of water.

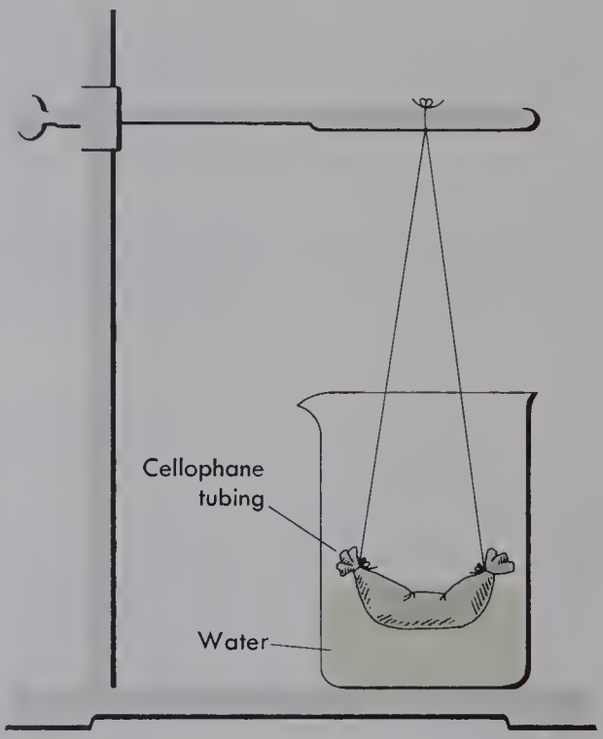


Fig. 3.12 Dialysis. The pores in a piece of cellophane are so small that macromolecules (e.g. starch, egg white) are retained inside while small molecules and ions (e.g. glucose, Na^+, Cl^-) pass out readily into the surrounding water. Dialysis is the separation of macromolecules from small molecules and ions.

Separating mixtures by column chromatography

The biologist is especially interested in colloids because a large proportion of the molecules found within living cells are **macromolecules,** that is, molecules so large that they form colloids. As we shall see in the next chapter, there are several categories of macromolecules within the cell and for some of these, e.g. proteins, a wide variety of specific types. The successful analysis of the chemistry of cells has depended in large measure on the development of techniques for separating these macromolecules from each other. Slight differences in size, shape, electrical charge, and solubility in different solvents must be exploited to accomplish these separations. Figure 3.13 shows a device, called a chromatographic column, used to separate closely related macromolecules (in this case, proteins) from each other.



Fig. 3.13 Separating macromolecules by column chromatography. A mixture of protein molecules in solution drips onto the top of the chromatographic column on the right. The packing material in the column separates the molecules according to size. As the isolated proteins leave the bottom of the column, they are collected in separate test tubes. (Courtesy of Instrumentation Specialities Company, Inc.)



Fig. 3.14 Chromatographic separation by size of molecule. The column is filled with semisolid beads which will admit small molecules (color), but not large molecules (gray) into their interior. Thus, when a mixture of the two enters the column (top), the small molecules become distributed in a larger volume of solvent than the large molecules. As the solvent moves down through the column, the large molecules are carried along more quickly than the small ones.

The column is filled with some solid material which will retard the passage through it of certain molecules more than others. For example, if the column is filled with a negatively charged solid, and a mixture containing both positively and negatively charged molecules is run through it, the positively charged molecules will be retarded in the column. This occurs because of the attraction of opposite electrical charges. By thus exploiting differences in electrical charge, the mixture of molecules can be at least partially purified.

On the other hand, if the column is filled with semisolid beads which will admit small molecules into their interior but not large ones, one can separate a mixture of molecules on the basis of their size. Upon adding such a mixture at the top of the column, the small molecules are able to distribute themselves in a larger volume than is available to the large molecules (Fig. 3.14). Consequently, the large molecules move more rapidly through the column and in this way the mixture can be separated (fractionated) into its components.

We now turn our attention to the general topic of the molecules from which living things are made.

EXERCISES AND PROBLEMS

1. Which of the following substances are composed of molecules: (a) oxygen, (b) water, (c) sodium chloride, (d) glucose, (e) steel?
2. Distinguish between an atom and an ion.
3. What ion is produced by all acids?
4. A mole of methane (CH_4) weighs how many grams?
5. Give an example of a strongly electropositive element.
6. Write electron-dot formulas for the molecules of (a) water, (b) ammonia, (c) methane, (d) ethane (CH_3CH_3).
7. Summarize the differences between mixtures and compounds.
8. Distinguish between organic and inorganic compounds.
9. Which of the following elements would you expect to react with chlorine: (a) hydrogen, (b) neon, (c) sodium, (d) fluorine, (e) calcium, (f) carbon?
10. Chemical analysis shows that the ratio of C atoms to H atoms in a substance is 1 to 3. The molecular weight of the substance is 30. What is the molecular formula?
11. Show by means of electron-dot formulas the combining of a lithium atom (3 electrons) with a fluorine atom (9 electrons).
12. A student needs 200 ml of 0.1 *M* NaOH solution. What weight of solid is used?
13. What is the molecular weight (MW) of acetic acid (CH_3COOH)?
14. When methane (CH_4) burns in oxygen, carbon dioxide and water are the products. Write a balanced equation to represent this chemical change.
15. What volume of 0.4 *M* hydrochloric acid will neutralize 200 ml of 0.2 *M* NaOH solution?
16. What is the most abundant metal in the crust of the earth?
17. Convert 98.6° F into °C.
18. Write a structural formula for butane (C_4H_{10}).
19. Write a different structural formula for butane.
20. What term is used to describe these two compounds?
21. Distinguish between electron, proton, and neutron.
22. Write an electron-dot formula for carbon tetrachloride (CCl_4).
23. Distinguish between isomers and isotopes.

REFERENCES

Frieden, E., "The Chemical Elements Of Life," *Scientific American,* July, 1972.

Additional information on the topics discussed in this chapter can be found in any good textbook on introductory chemistry, such as:

Mahan, B. H. *College Chemistry,* Addison-Wesley, Reading, Massachusetts, 1966.

A small book that covers topics in chemistry and physics, selected and developed with the needs of biology students in mind, is:

Baker, J. J. W., and G. E. Allen, *Matter, Energy, and Life: An Introduction for Biology Students,* 2nd ed., Addison-Wesley, Reading, Massachusetts, 1970.

4 THE CHEMICAL BASIS OF LIFE: THE MOLECULES OF LIFE

Carbohydrates

Fats

Proteins

Nucleic Acids

Vitamins

Minerals

Water

The same basic chemical principles apply to matter found in living as well as nonliving things. Matter found in living things does, however, have certain distinguishing characteristics. One is that the number of different elements found in living matter is considerably smaller than the number found in nonliving matter. Furthermore, living things are largely composed of carbon, hydrogen, nitrogen, and oxygen. Of these elements, only oxygen is plentiful in the nonliving world.

Perhaps the one element which, more than any other, is characteristic of living matter is carbon. Because of the ability of carbon atoms (1) to unite with one another and thus form long chains and (2) to form double covalent bonds, they participate in the formation of an almost infinite variety of molecules. Such variety is essential for such an incredibly complex organization of matter as a living thing. It is no wonder, then, that molecules containing carbon form the very basis of life itself. In fact, the chemistry of carbon compounds is called **organic chemistry** because these compounds are almost exclusively associated with life processes.

Organic chemistry is the chemistry of carbon compounds

It is fortunate for our understanding of the organization and functioning of living things that the almost unlimited numbers of organic compounds do fall into a reasonable number of distinct categories. As far as quantity is concerned, organisms are composed mainly of water, proteins, fats and other lipids, carbohydrates, nucleic acids, and minerals (Fig. 4.1). Of these, water and minerals are inorganic. The other substances are all organic.

4.1 CARBOHYDRATES

Carbohydrates have the general formula $(CH_2O)_n$

Carbohydrates are essential to life. Almost all organisms use them as a fuel, exploiting their rich supply of potential energy to maintain life. Carbohydrates also serve as an important structural material in some animals and in all plants. Carbohydrates are made of molecules containing carbon, hydrogen, and oxygen atoms with the ratio of hydrogen atoms to oxygen atoms always 2:1. In view of this, can you imagine how the word carbohydrate came to be used for these compounds?

Many carbohydrate molecules are extremely large, possessing molecular weights of 500,000 or more. Fortunately, these **macromolecules** are composed of simpler, repeating units, and this makes them relatively easy to study. The repeating units are called sugars.

1. Sugars

Glucose and its isomers are monosaccharides

The most important sugar is **glucose.** It has the molecular formula $C_6H_{12}O_6$, but as is so often the case in organic chemistry, the molecular formula is not sufficient to describe the molecule fully. There are other common sugars that have precisely the same molecular formula and thus are **isomers** of glucose. Glucose can, however, be distinguished from these by its structural formula (Fig. 4.2). Five of the six carbon atoms and one of the oxygen atoms form a flat ring. The hydrogen atoms and other groups extend above and below the plane of this ring.

Molecular composition of the human infant	
Water	66%
Proteins + nucleic acids	16%
Fats and lipids	12.5%
Ash (minerals)	5%
Carbohydrates	0.5%
Vitamins	trace

Fig. 4.1 In the human infant, water represents almost two-thirds of its weight.

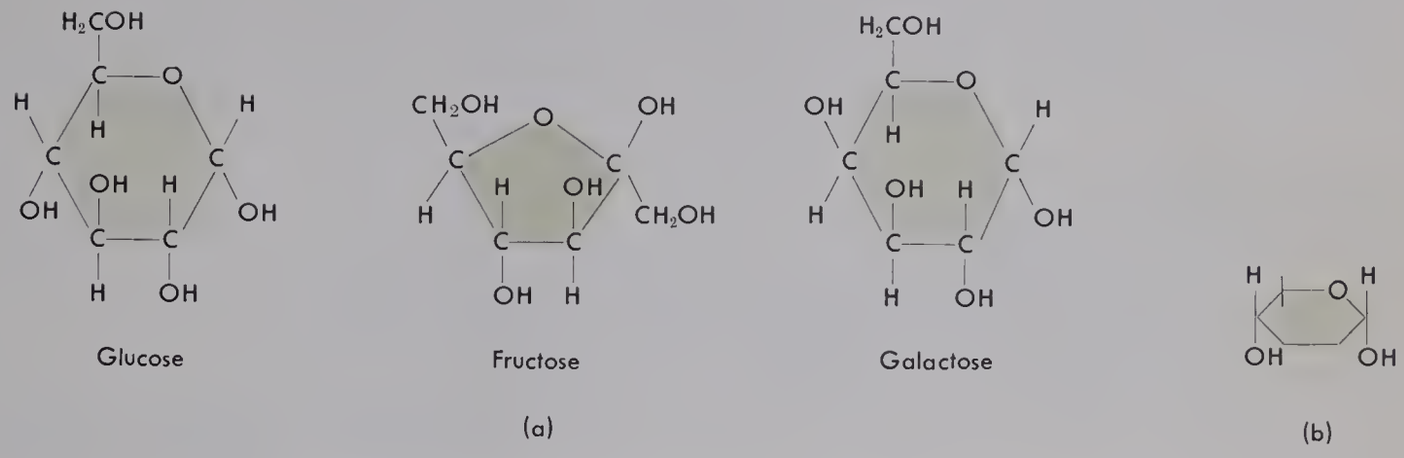


Fig. 4.2 (a) Structural formulas of glucose and two of its isomers. (b) Simplified version of the glucose molecule.

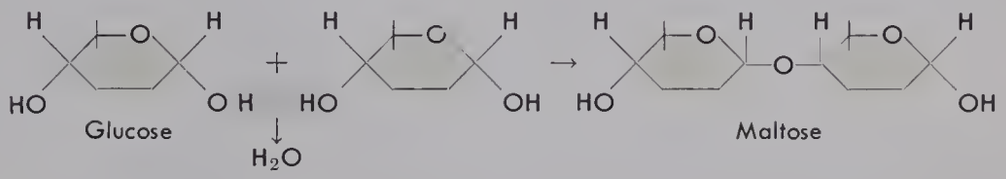


Fig. 4.3 Synthesis of maltose.

The great importance of glucose to life is that it serves as the basic, transportable form of fuel for the organism. It is soluble and thus easily transported by body fluids. In humans, glucose is actually referred to as "blood sugar." Its concentration in the blood must be maintained within narrow limits or serious disturbances will result. Glucose is a starting material for cellular respiration, the process from which most organisms derive the energy they need to run their life activities.

Disaccharides

Two glucose units can be linked together by an oxygen bridge between them (Fig. 4.3). This is formed by the removal of a molecule of H_2O. A molecule of **maltose** results. It is a double sugar or **disaccharide,** with the molecular formula

$$C_{12}H_{22}O_{11} \quad (2C_6H_{12}O_6—H_2O).$$

Maltose is produced when starch is digested.

The sugar that you put in your coffee or on your breakfast cereal, **sucrose,** is also a disaccharide. It is formed by the linking together of a glucose unit and a unit of its isomer **fructose.** (What, then, is the molecular

formula of sucrose?) Our chief source of sucrose is sugar cane and sugar beets. Most higher plants transport carbohydrate as sucrose. It is very soluble in water and can thus be easily transported in sap.

2. Starches

Polymers are macromolecules built from repeating units

Starches are large carbohydrate molecules made up of chains of glucose units (Fig. 4.4). We use the term **polymer** to describe chainlike molecules of this sort. The "links" in the chain are **monomers.** Most natural starches contain both unbranched chains of several hundred glucose units (amylose) and branched chains totalling more than a thousand glucose units (amylopectin). Because of the many sugar units present in starches, they are called **polysaccharides.**

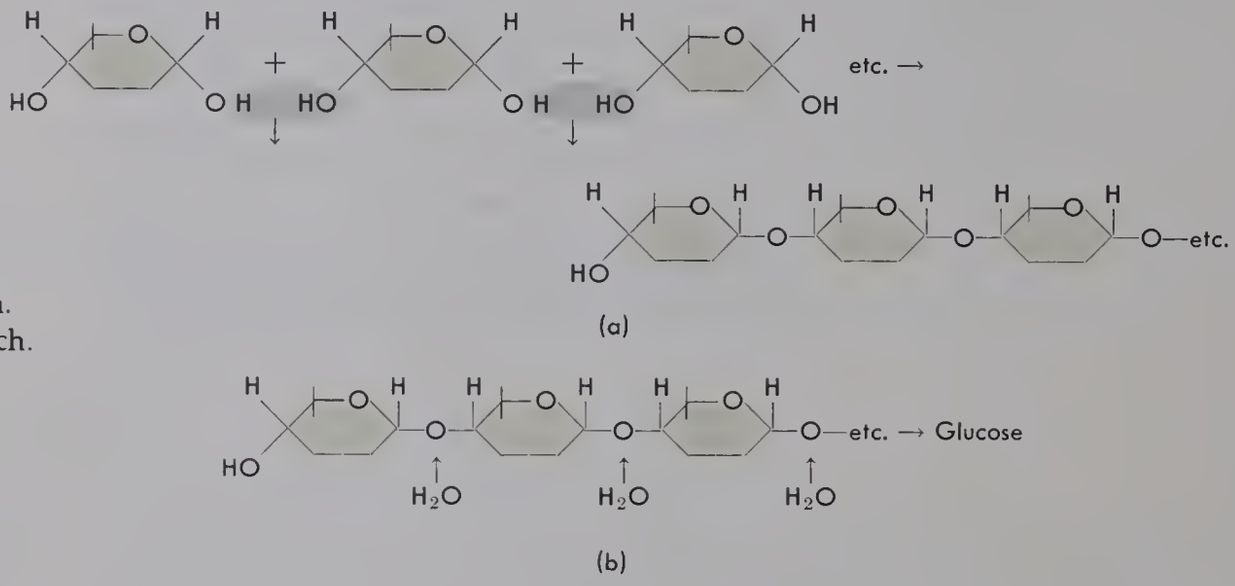


Fig. 4.4
(a) Synthesis of starch.
(b) Hydrolysis of starch.

Starches are important because they serve as a storage form of sugar. Surplus sugar can be converted into starch, which is insoluble, and stored. This takes place in both plants and animals. Man's greatest source of carbohydrates is plant starch. Most of the world's population satisfies its energy needs with the starches of rice, wheat, corn and potatoes. Before starches can be absorbed into the body, however, they must be digested. This simply means that the long chains are broken back down into their sugar links. This process requires both water and starch-digesting enzymes called **amylases.** With the aid of amylases, water molecules enter at each oxygen bridge, re-forming free sugar molecules (Fig. 4.4). The process of breaking up a molecule by inserting water is called **hydrolysis.**

Hydrolysis

Amylases hydrolyze starches into the disaccharide maltose. Another enzyme, maltase, hydrolyzes the maltose molecule into two glucose molecules.

Glycogen is the starch synthesized by animals

When man takes in more glucose than he needs, he converts some of the excess to starch. This starch, as well as that produced by other animals, differs somewhat in its properties from plant starch. For this reason it is often given a special name, **glycogen.** The main difference between glycogen and plant starch lies in the pattern of branching. There seem to be no

straight chain molecules in glycogen like the amylose chains in plant starches.

Man stores glycogen in his muscles and in his liver. In these locations, it is quickly available for breakdown into glucose and subsequent energy production. Glycogen is not, however, the major storage form of energy in man. We depend on fat deposits for most of our energy reserves. There are some animals, though, that do rely almost entirely on glycogen stores. These are apt to be animals such as mussels (mollusks) that regularly undergo periods of oxygen shortage. The advantages of glycogen as a source of energy when oxygen is lacking will be discussed in Chapter 8.

3. Cellulose

Cellulose is the most abundant organic molecule on our planet

Cellulose is another important polysaccharide. It is universally found in plants and is, in fact, their chief structural material. The rigidity of plants is a consequence of the large quantities of cellulose they produce. Wood is chiefly cellulose. Cotton and the paper upon which this book is printed are almost pure cellulose. The extensive use of cellulose as a structural material in plants makes it without doubt the most prevalent organic molecule on earth.

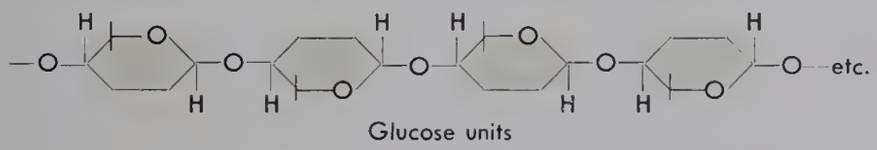


Fig. 4.5 Structure of cellulose. Compare its structure with that of starch (Fig. 4.4).

Like amylose molecules, cellulose molecules consist of long chains of glucose units. A single cellulose molecule may contain over three thousand of these. Cellulose differs from starch, however, in the way the glucose molecules are attached to one another. In starches, the glucose molecules are all oriented in the same fashion. In cellulose the orientation of the glucose units alternates from one glucose molecule to the next (Fig. 4.5).

Cows depend on bacteria to digest the cellulose they eat

This difference in molecular structure is sufficiently great that amylases are unable to hydrolyze cellulose. Cellulose can be digested by **cellulases,** which are produced by certain bacteria, protozoans, and a few invertebrates such as terrestrial snails and some insects. What of the cows, rabbits, and termites which seem to thrive on cellulose? In each of these cases, the cellulose digestion is accomplished by microorganisms living within the animal's digestive system. The rumen of cows and the caecum of rabbits are large pouches in which food is stored while bacteria digest cellulose with their cellulases. Both the bacteria and their mammalian host then absorb the sugars produced. Termites also depend upon microorganisms to carry out the same function in their gut.

Although cellulose is not digested by man, it does form a valuable part of his diet by providing bulk and thus giving his digestive tract something to work on. Preparations of purified cellulose are sometimes eaten in order to stimulate movement of the bowels and cure constipation.



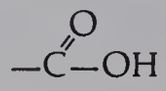
Fig. 4.6 Burning Brazil nut.

4.2 FATS

Fat molecules are also composed solely of carbon, hydrogen, and oxygen atoms. In contrast to carbohydrates, however, the ratio of hydrogen atoms to oxygen atoms is a great deal higher than 2:1. Tristearin, to take a common example of a fat, has the molecular formula $C_{57}H_{110}O_6$.

The high proportion of hydrogen in a fat molecule tells us that the molecule is much less oxidized than a carbohydrate molecule. This means that a given weight of fat stores a good deal more energy than the same weight of carbohydrate. Pound for pound, fats provide the most concentrated energy reserve available to the organism. Figure 4.6 shows a Brazil nut burning like a candle, thanks to its rich oil stores.

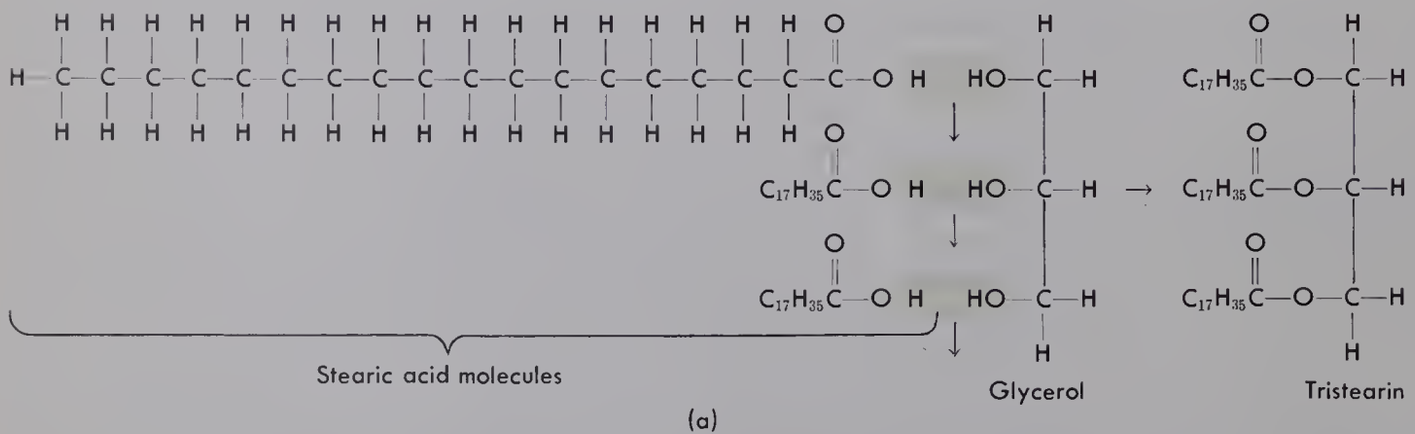
The fat molecule is made up of four parts: a **glycerol** molecule and three molecules of **fatty acids.** Each of the three —OH groups on the glycerol molecule is capable of reacting with the



The structure of fat molecules

group of a fatty acid molecule. A molecule of water is removed in this process, too, and the fatty acid becomes attached to the glycerol molecule (Fig. 4.7). The three fatty acids in a single fat molecule may be all alike or they may be different. They may contain as few as four carbon atoms or as many as 24. Curiously enough, the number of carbon atoms in the fatty acid is almost always an even number. 16-carbon (palmitic acid) and 18-carbon (stearic acid) fatty acid molecules are the most common in fats.

Just as polysaccharides can be hydrolyzed into their constituent sugar units, so fats can be hydrolyzed into glycerol and fatty acids (Fig. 4.7). Water is necessary, of course, as well as an enzyme called a **lipase.** The hydrolysis of fat is an important step in the digestive process.



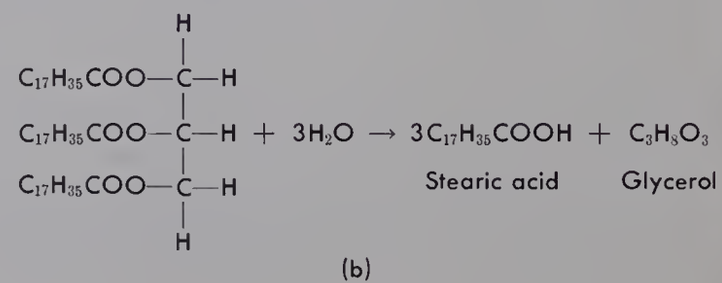


Fig. 4.7 (a) Synthesis of tristearin, a fat. The three stearic acid molecules are bracketed. (b) Hydrolysis of tristearin.

Unsaturated fats contain one or more double bonds

Some fatty acids have one or more double bonds in the molecule (Fig. 4.8). Fats formed from these molecules are known as unsaturated fats. As they are likely to melt at temperatures below room temperature, we call them oils. Cottonseed oil and olive oil are two examples. These oils should not be confused with oils derived from petroleum. Their chemical nature is quite different.

In general, animal fats are saturated; plant fats unsaturated. There is a growing body of evidence that a diet rich in saturated fats predisposes one to the development of diseases of the heart and circulatory system. Unsaturated fats, on the other hand, seem not to have this effect.

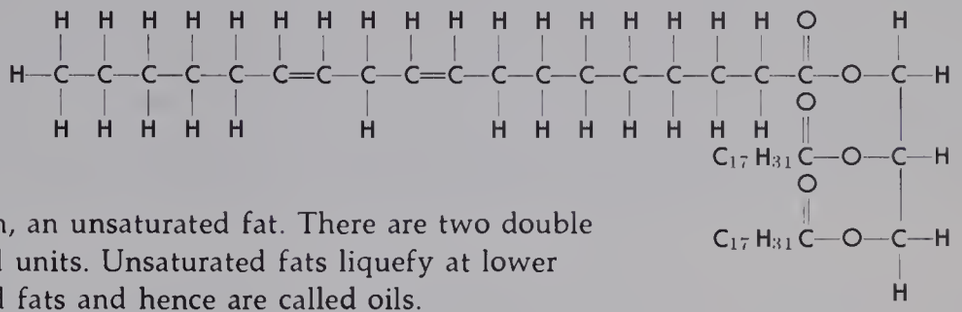


Fig. 4.8 Structure of trilinolein, an unsaturated fat. There are two double bonds in each of the fatty acid units. Unsaturated fats liquefy at lower temperatures than do saturated fats and hence are called oils.

Phospholipids are a major component of the membranes of cells

Fats are insoluble in water and before they can be transported in the blood, they must be converted into a form that will mix with water. One way in which this is done is to substitute a phosphorus-containing molecule for one of the three fatty acid molecules. The resulting substance, a **phospholipid,** mixes with water and thus can be transported in the watery environment of the body. Phospholipids also play an important structural role in the organism. In close association with protein, they participate in forming the membranes from which many cell structures are built.

There is another way in which fats can be converted into a form which will mix with water. This is to emulsify them. **Emulsions** are colloids containing colloidal size droplets of one liquid suspended in another. Homogenized milk is an example of an emulsion of fat (cream) in the watery portion ("skim milk") of the milk. In order to keep the droplets of an emulsion from meeting and combining, it is necessary to coat them with a third substance, an emulsifying agent. Soaps and synthetic detergents are effective emulsifying agents for fats. We use these substances to emulsify fat deposits on our bodies and dishes. In the form of an emulsion, the fat can then be easily rinsed away.

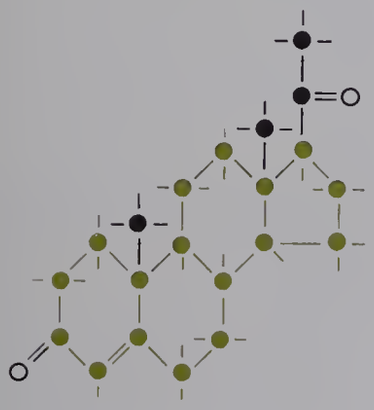


Fig. 4.9 Progesterone, an important sex hormone in women. Like all steroids, it is constructed from a skeleton of 17 carbon atoms (shown in color). Each short dash represents a hydrogen atom.

A good deal of the fat we eat is first emulsified in the digestive tract before it is used by the body. The body's emulsifying agent is a fluid called bile which is secreted into the intestine by the liver. The active ingredients in bile are substances called bile salts. They are members of a large group of closely related chemical compounds, the **steroids.** All steroids contain a skeleton of seventeen carbon atoms organized into four rings (Fig. 4.9). Each different steroid has its own side groups attached to several points on the skeleton. The most abundant steroid in the human body is choles-

terol (about one-half pound per person). From it are synthesized the bile salts, one of our vitamins, and a number of important hormones. All the sex hormones, of which progesterone is one (Fig. 4.9), are steroids.

4.3 PROTEINS

Composition of proteins

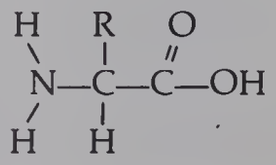
Proteins are among the most complex of all the organic compounds. They are made up of macromolecules which contain carbon, hydrogen, oxygen, and nitrogen atoms. Sulfur atoms are usually present, too. Certain proteins contain phosphorus or some trace metal element, such as iron or copper, in addition to the other elements.

Size of proteins

Most protein molecules are so large and complex that it has not yet been possible to learn much about their structure except their size and, in some cases, their molecular formula. Beta-lactoglobulin, a protein with a relatively small molecule, has the molecular formula $C_{1864}H_{3012}O_{576}N_{468}S_{21}$. Its molecular weight is about 42,000. Many proteins are even larger than this. Hemocyanin, an oxygen-carrying pigment found in some crustaceans, has a molecular weight greater than six million.

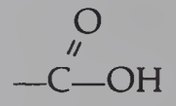
At first glance, it might seem a hopeless task to learn more about the structure of such enormous molecules. Fortunately, the problem is not quite so insurmountable as it might seem. Despite their great size, protein molecules are built up in an orderly way. They are polymers consisting of long chains of relatively simple monomers called amino acids. There are twenty different kinds of amino acids commonly found in proteins. Each of these is built according to the plan:

Proteins are polymers



Twenty different amino acids serve as the monomers of proteins

The $—NH_2$ group is called the amino group. The



group is the acid group. The chemical nature of R is different for each of the different amino acids. In every case, however, the R group is relatively simple and its structural formula is known (Fig. 4.10).

Amino acids are linked together by the removal of water molecules from between them (Fig. 4.11). Crosslinks between chains are found where sulfur-containing amino acids are present. The chains are also folded. Figure 4.12 shows a model of the arrangement of the amino acid chain in the myoglobin molecule. This molecule is one-quarter of the size of the hemoglobin molecule, having a molecular weight of 17,000.

The "primary" structure of a protein is its sequence of amino acids

Although even the most complex proteins are made up of simple, repeating units of amino acids, we are still a long way from working out detailed structural formulas for them. In fact, it was not until 1954 that the structural formula of the first protein was established. The protein was

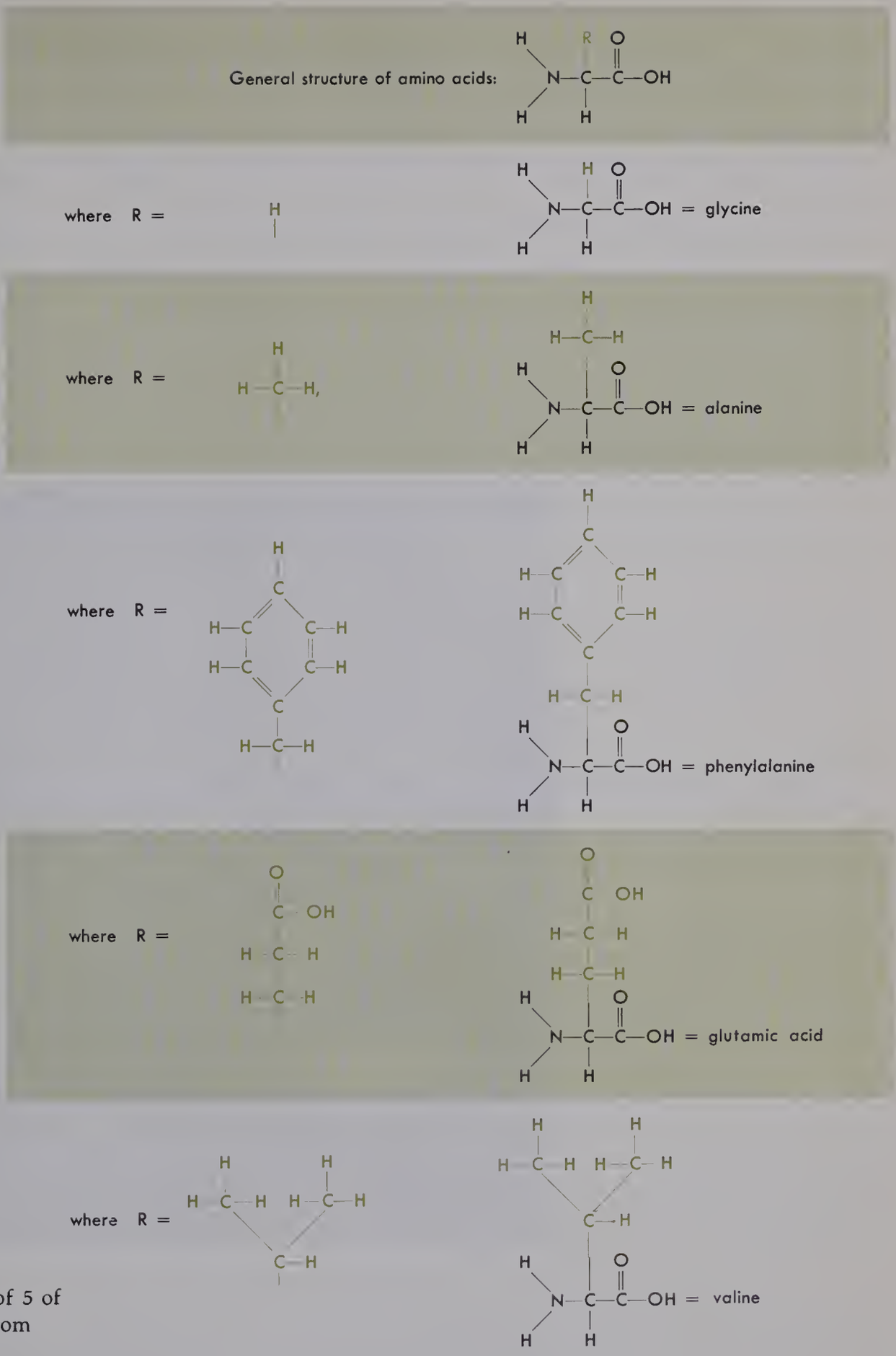


Fig. 4.10 Structures of 5 of the 20 amino acids from which proteins are synthesized.

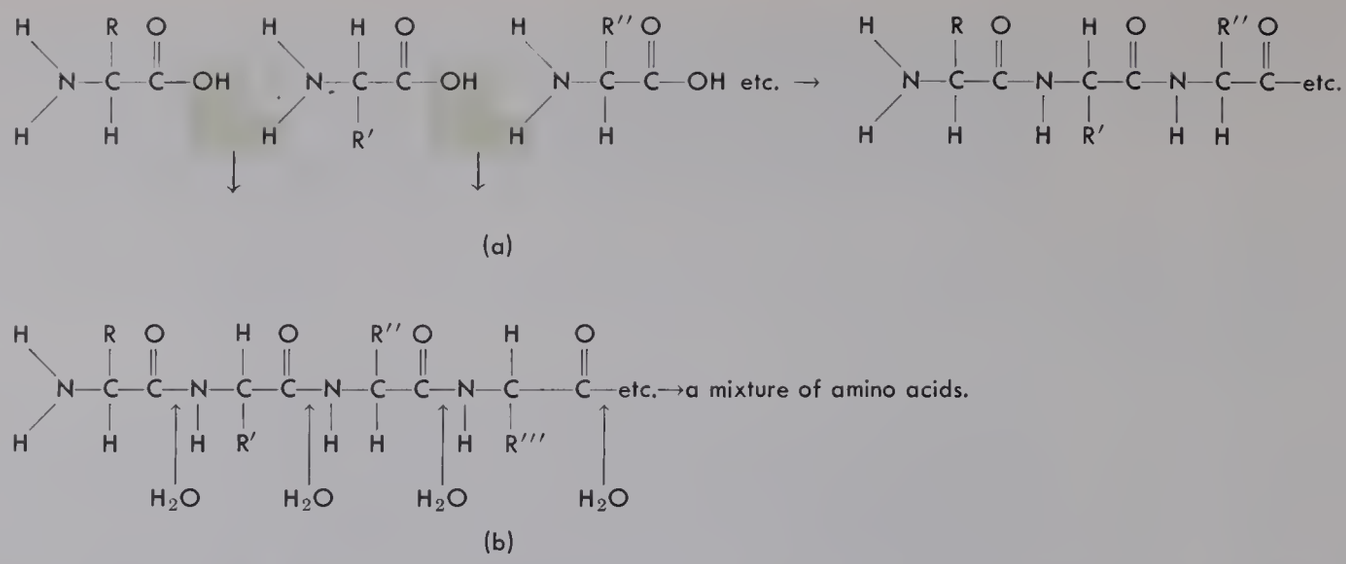


Fig 4.11 (a) Synthesis of protein. (b) Hydrolysis of protein.



Fig. 4.12 Three-dimensional model of the myoglobin molecule. About 150 amino acids are present in the single, folded chain. The dark sphere represents the iron atom at the center of the prosthetic group, heme. The light sphere represents a molecule of water. (Courtesy of Dr. John C. Kendrew.)

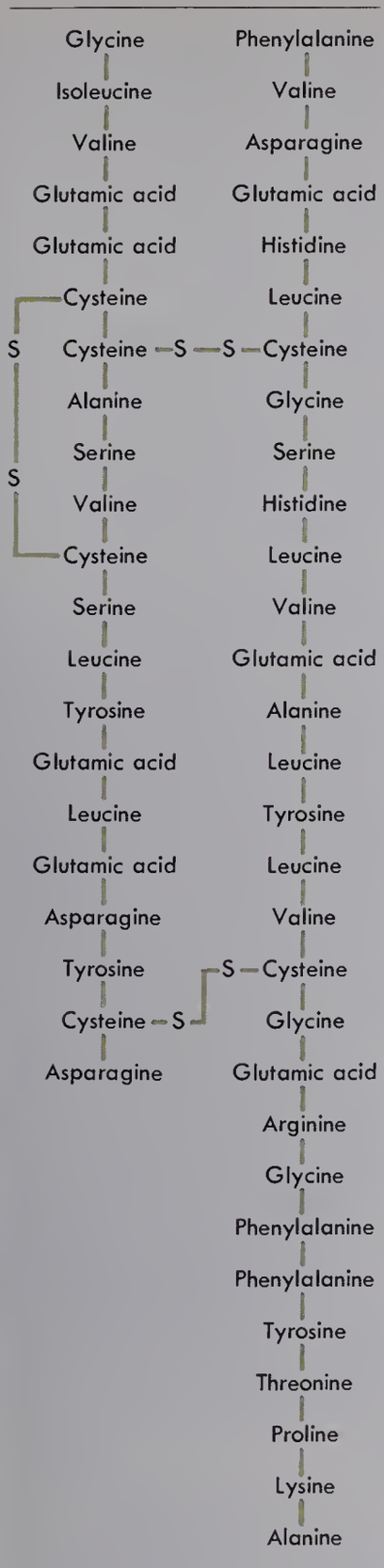


Fig. 4.13 Sequence of amino acids in the insulin molecule. The insulin molecule consists of two polypeptide chains held together by sulfur bridges.

insulin, one of the most important hormones in our body. Its molecular formula, $C_{254}H_{377}N_{65}O_{75}S_6$, shows that its molecule is small compared with most proteins. Nevertheless, it took Dr. Frederick Sanger and his colleagues at the University of Cambridge in England ten years (1944–1954) to work out the sequence of amino acids and the locations of the crosslinks in this protein. Using clever, but tedious, steps of chemical degradation, they determined the exact sequence of the 51 amino acids present in the molecule (Fig. 4.13). Since their discovery, other workers have applied similar techniques in an effort to work out the structure of still other proteins.

Knowledge of the exact sequence of amino acids in a protein naturally raises the possibility of synthesizing the protein in the "test tube." The task of assembling dozens of amino acids in a precise sequence is a formidable one, but it has been accomplished for insulin. And, in 1969, the enzyme ribonuclease, which is also a protein, was successfully synthesized. There are 124 amino acids (in a single chain) in the ribonuclease molecule.

Proteins, like starches and fats, are degraded by hydrolysis. The peptide linkages are opened with the insertion of a molecule of water at that point (Fig. 4.11). This breaks the protein molecule into shorter chains of amino acids called **polypeptides.** Finally the polypeptides are completely hydrolyzed into their constituent amino acids.

Life as we know it could not exist without proteins. They are the chief molecules out of which living cells are made. Some protein molecules are dissolved or suspended in the watery contents of the cell. Others are incorporated into various structures of the cell.

Many cell proteins are found chemically united with other kinds of molecules. These proteins are called **conjugated proteins.** Protein-lipid, protein-nucleic acid, protein-carbohydrate, and protein-pigment combinations all play important roles in living cells. All enzymes are proteins and many of them are conjugated with smaller molecules **(prosthetic groups)** such as metal-containing pigments and vitamins.

Proteins are also found outside of cells. These extracellular proteins are important supporting and strengthening materials in animals. (Plants differ in this respect from animals. Their cells are supported and strengthened by what extracellular, organic molecules?) Bone, cartilage, tendons, and ligaments are all examples of supporting structures which contain substantial quantities of extracellular proteins, such as collagen.

Although we can make statements about the general kinds of proteins found in different organisms, it is important to realize that these proteins may not be exactly alike. Every species manufactures proteins unique to that species. Furthermore, even the individuals within a species may have some protein molecules that are absolutely unique. With the exception of identical twins, we are quite sure that no two human beings, for example, have precisely the same proteins.

At first glance, it seems unbelievable that each individual now living on earth has certain protein molecules unique to him. When you appreciate the great complexity and possibilities for variety in protein molecules,

however, this specificity seems more reasonable. Just think for a moment of the large number of words in an English unabridged dictionary. Every one of these is made up from an alphabet of 26 letters. Proteins are made up from an "alphabet" of about twenty amino acids. Then think of the dictionaries in other languages that also use the Roman alphabet. Consider, too, that it is a rare word indeed that contains more than twenty letters. A single protein molecule, on the other hand, may contain thousands of amino acids.

The three-dimensional shape of a protein is known as its "tertiary" structure

The amino acids in proteins are not arranged in just one dimension as are the letters in a word and as they are shown in Fig. 4.13. Instead, the chains of amino acids are folded into complex, three-dimensional shapes (Fig. 4.12). This folding occurs because of interaction between certain amino acids in the chain. For example, the sulfur (—S—S—) bridges that form between the cysteine units in the chain (Fig. 4.13) bring these links in the chain close together.

Denaturation of proteins

The importance of the three-dimensional structure of proteins is nicely illustrated by the phenomenon of **denaturation.** Proteins are quite sensitive to a variety of chemical and physical agents. When exposed to these agents, they may lose their characteristic pattern of folding. This is denaturation. It can occur under conditions that do not affect the actual peptide linkages themselves. Nevertheless, the biochemical properties of the protein may be completely altered. For example, denaturation destroys the activity of enzyme molecules. When the white of an egg cooks, the protein (albumin) is undergoing denaturation. When more gentle methods of denaturation are used, the process may be reversible. In such cases, removal of the denaturing agent allows the protein to regain its original three-dimensional shape and full chemical activity.

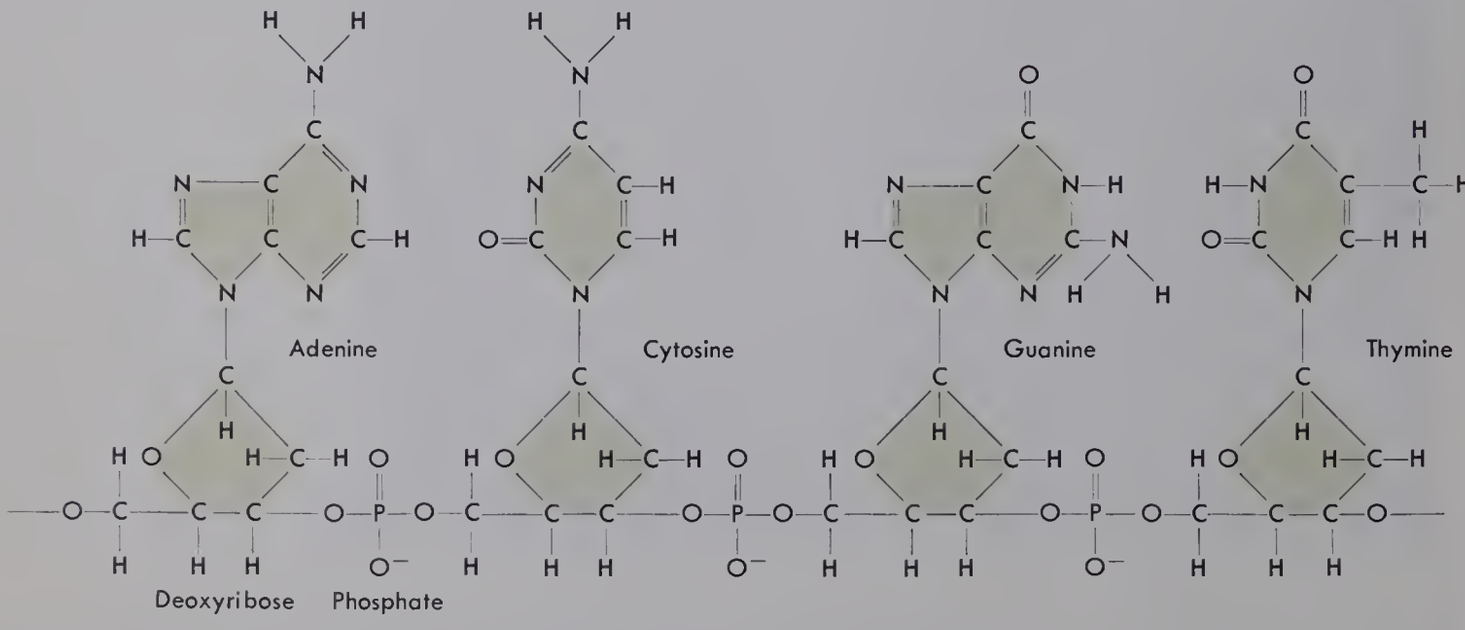


Fig. 4.14 Molecular organization of DNA. In RNA the pyrimidine uracil is found in place of thymine.

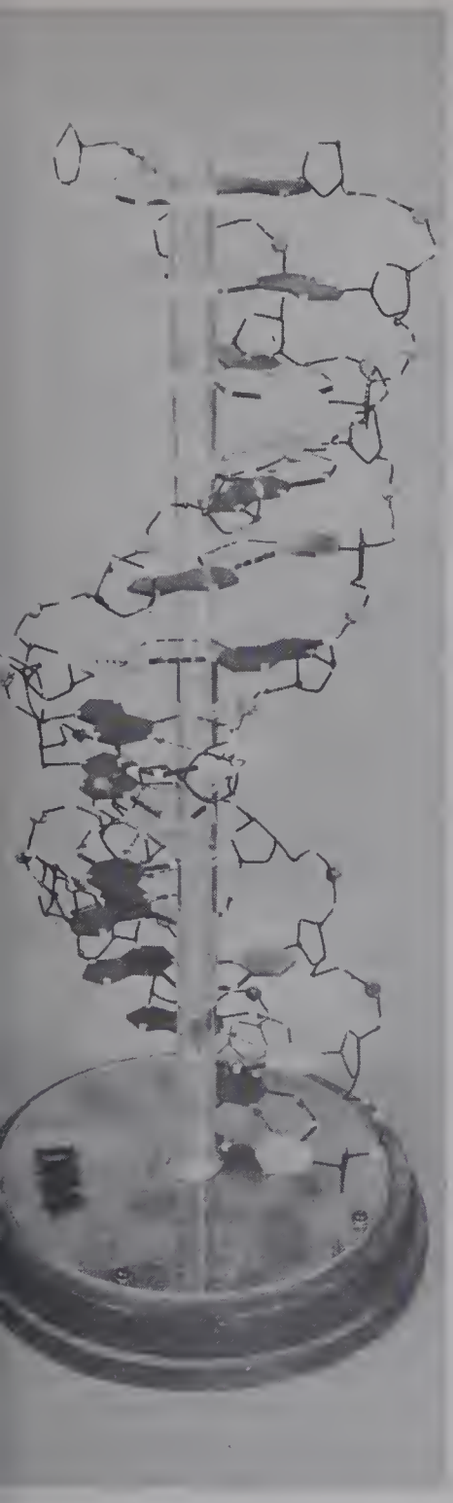


Fig. 4.15 The Watson-Crick model of DNA structure. The small spheres represent phosphate groups; the open pentagons represent deoxyribose. The solid planar structures represent the purine and pyrimidine bases. (Photo courtesy of Dr. Donald M. Reynolds.)

Proteins can also be used by living organisms as a source of energy. If more protein is consumed in the diet than is needed to satisfy the structural needs of the body (growth and repair), the excess can be used as fuel. After the protein is hydrolyzed into its constituent amino acids, the nitrogen-containing, or amino, portion of these molecules is removed. In man, this process, called **deamination,** occurs chiefly in the liver. The non-nitrogen-containing residue is then oxidized in the same way as carbohydrate and fat materials.

4.4 NUCLEIC ACIDS

Nucleic acid molecules are even larger than protein molecules, with molecular weights that run into the billions. They, too, are polymers. The monomers of nucleic acid molecules are called **nucleotides.** Each consists of three subunits: a sugar, a phosphate, and a nitrogenous base. The sugar and phosphate groups alternate as links in the chain. The nitrogenous bases project out from the axis of the chain, with one base attached to each sugar link (Fig. 4.14).

There are two distinct kinds of nucleic acid. One is called deoxyribonucleic acid or DNA. The other is ribonucleic acid or RNA. DNA is found localized within the control center of the cell, the nucleus. Some RNA is also found there, but more of it is found in the surrounding cell contents, the cytoplasm.

RNA gets its name from its sugar unit, a five-carbon sugar called ribose. Attached to each ribose unit is one of four possible nitrogenous bases, cytosine, uracil, adenine, and guanine (C, U, A, and G). The first two of these bases are single-ring substances called pyrimidines. The second two are double-ring substances called purines.

The sugar unit in DNA is deoxyribose. As its name suggests, it has one less oxygen atom than ribose. The pyrimidines, cytosine and thymine, and the purines, adenine and guanine (C, T, A, and G), are the bases found in DNA.

Normally, DNA differs from RNA in another important respect. Its molecule is made up of not one, but two chains of alternating sugars and phosphates. These two chains are twisted around each other something like a double spiral staircase (Fig. 4.15). At each sugar, a purine or pyrimidine projects into the "stairwell." Here it joins with the pyrimidine or purine projecting out from the opposite staircase. Weak forces of attraction between these bases are sufficient to hold the two chains together.

The most interesting thing about this structure is that there is not room enough across the "stairwell" for two purines to fit at the same level, and there is too much room for two pyrimidines to meet and bond together. Consequently, a purine on one chain can be paired only with a pyrimidine on the other. Furthermore, the location of the bonds is such that the purine adenine can pair only with thymine. Similarly, guanine always pairs with cytosine. Thus the two interlocking strands of a DNA molecule are complementary to each other. In a real sense, one is the

"negative" of the other. If we know the sequence of bases on one strand, we know the sequence of bases on the other.

In recent years many biologists have turned their attention to the nature and role of DNA and the three types of RNA that are found within cells. Their interest stems from the knowledge that within these molecules resides the information that controls the structure and activities of all organisms and the machinery for translating this information. They see DNA as a master blueprint, written in the code of purine-pyrimidine pairs, for the building and operating of a living organism. They see each of the three major types of RNA as playing an essential role in the execution of the instructions coded within the DNA molecules. (Some of the discoveries that have led to these ideas will be discussed in the next chapter and in Chapter 19.)

Perhaps one day the fundamental nature of life itself can be understood in molecular terms; it begins to look as though the study of the nucleic acids will bring us closer to this goal than ever before.

4.5 VITAMINS

The carbohydrates, fats, proteins, and nucleic acids are the most prevalent organic molecules found in living things. There are others such as organic acids, alcohols, and steroids, that play important roles, too. These are all small molecules, however, and do not contribute to the fixed, structural components of the cell. Furthermore, almost all of them can be synthesized by the organism from one or more of the major kinds of molecules that we have been discussing.

The few organic molecules that an organism cannot manufacture from carbohydrate, fat, protein, or nucleic acid sources are called vitamins. With a few exceptions, green plants have no vitamin requirements as they manufacture every organic molecule they need. Among these, however, are substances that are vitamins for nongreen plants, microorganisms, and animals. It is important to realize that the term vitamin does not refer to a particular chemical group. The only way in which the various vitamins are similar to one another is that (1) they are all organic, (2) they are not used as an energy source or for construction of the cell, and (3) the organism cannot synthesize them from the standard foods in its diet. Note, too, that what may be a vitamin for one organism is not necessarily a vitamin for another.

Characteristics of vitamins

Vitamins are needed in only very small quantities. Years of research have shown that some of them, at least, are incorporated into enzymes. These vitamins are conjugated as a "prosthetic group" with the protein part of the enzyme. In view of this role, it is not surprising that their presence is vital to the welfare of the organism. If it cannot synthesize them, then it must include them in its diet. If it fails to do so, a **deficiency disease** may result (Fig. 4.16).

It has been suspected for a long time that certain diseases result from special deficiencies in the diet. Years ago on long ocean voyages, when

VITAMIN	DEFICIENCY DISEASE	SOURCES	OTHER INFORMATION
A	Night blindness	Milk, butter, fish liver oils, carrots, and other vegetables	Precursor in the synthesis of the light-absorbing pigments of the eye Stored in the liver Toxic in large doses
Thiamine (B_1)	Beriberi Damage to nerves and heart	Yeast, meat, unpolished cereal grains	Coenzyme in cellular respiration
Riboflavin (B_2)	Inflammation of the tongue Damage to the eyes General weakness	Liver, eggs, cheese, milk	Prosthetic group of flavoprotein enzymes used in cellular respiration
Nicatinic acid (Niacin)	Pellagra (Damage to skin, lining af intestine and perhaps nerves)	Meat, yeast, milk	Converted inta nicotinamide, a precursar of NAD and NADP—two impartant coenzymes for REDOX reactions in the cell
Folic acid	Anemia	Green leafy vegetables Synthesized by intestinal bacteria	Used in synthesis of coenzymes of nucleic acid metabolism
B_{12}	Pernicious anemia	Liver	Each malecule contains ane atom of cobalt.
Ascorbic acid (C)	Scurvy	Citrus fruits, tomatoes, green peppers	Coenzyme in synthesis of collagen
D	Rickets (Abnormal Ca^{++} and $PO_4^{\equiv}$ metabalism resulting in abnormal bone and tooth development)	Fish liver oils, butter, steraid-containing foods irradiated with ultravialet light	Synthesized in the human skin upon exposure to ultravialet light Toxic in large dases
E	No deficiency disease known in humans	Egg yolk, salad greens, vegetable ails	–
K	Slow clotting of the blaad	Spinach and ather green leafy vegetables Synthesized by intestinal bacteria	Necessary far the synthesis of prothrombin, an essential agent in the clotting of blood

Fig. 4.16 Some of the principal vitamins.

fresh fruits and vegetables might be unavailable for months at a time, sailors became prone to the disease **scurvy.** As early as 1771, Captain Cook made it a practice to carry limes on his voyages. Adding them to the diet of his crew prevented the appearance of scurvy and gave the British sailors a nickname they bear to this day: "limeys."

It was not until this century that scientists began to search for the specific chemical substances that would prevent or cure deficiency diseases. In 1915, the American nutritionists E. V. McCollum and M. J. Davis discovered that both a fat-soluble (vitamin A) and a water-soluble substance (vitamin B) were needed for proper growth in laboratory rats.

Since their discovery, other vitamins have been found. Vitamin A has turned out to consist of two different kinds of molecules. Vitamin B has

been found to consist of almost a dozen different substances. Today we place these latter in the "B-complex" family of vitamins. They do not, however, have any greater chemical similarities to each other than they do to any of the other vitamins.

Figure 4.16 lists the vitamins that we think are needed by man, the symptoms caused by a deficiency of these vitamins, rich sources of the vitamins, and some supplementary information. In the case of vitamin E (and also some other "vitamins" that are not included in this table), there is some doubt whether the substances are really vitamins for man. Their discovery has come about as a result of diet experiments in laboratory animals like the rat, and it is somewhat risky to apply to man discoveries made in other animals. We know, for example, that the laboratory rat can synthesize its own "vitamin C." (Thus, for the rat, it is not a vitamin.) If we need an external supply of a substance which the rat does not need, is it not possible that the reverse may also be true? In the case of vitamin E, no sound evidence of a deficiency disease in man has been found, although the substance is definitely present in the body (where it seems to participate in the metabolism of unsaturated fatty acids).

The discovery of the vitamins has created a great interest in the general subject of diet. This has caused deficiency diseases to become far less common than they used to be, at least in areas where the standard of living is reasonably high. Unfortunately, the great interest in vitamins has also had drawbacks. Many people now consume commercial vitamin preparations when there is absolutely no medical reason for doing so. Apparently, they feel that if a little of something is good, more must be better. This is not true of the vitamins. Not only does the annual expenditure in this country of millions of dollars on the purchase of vitamins represent a great waste of money, but it can lead to unpleasant complications. Excess intake of vitamin A or D is quite apt to produce disease symptoms. A glance at the list of vitamin sources in Fig. 4.16 should make it quite clear that a normal, well-balanced diet (including fresh fruits, vegetables, meat, and milk) provides all the vitamins that most of us need. Only in special circumstances do our diets need to be supplemented with vitamin preparations. In these cases, a doctor should be consulted and his advice followed.

4.6 MINERALS

Ionic compounds are also important to life

Although living organisms are distinguished by the complex array of organic molecules of which they are made, inorganic substances play an important part, too. Aside from C, H, O, N, and S, most of the elements found in living things are present in the form of ions of salts. These are the inorganic minerals of the body. Some of these minerals are quite insoluble in water and form solid deposits. Many organisms exploit these as supporting and protecting structures. Calcium carbonate ($CaCO_3$) is the principal constituent of the shells of mollusks. The bones of vertebrate skeletons contain calcium carbonate, large amounts of calcium phosphate [$Ca_3(PO_4)_2$] and some magnesium and fluoride ions. All these substances

provide rigid support and protection for soft tissues of the body. Inorganic ions also carry out other important roles in living things. We shall examine some of these in Chapter 6.

4.7 WATER

Water is the single most abundant molecule in living things. It represents some 60% of the weight of the adult human body and as much as 95% of the weight of such delicate forms as jellyfishes and embryos.

Water has several important properties that make it an ideal constituent of living things. One of these is that it remains liquid over the range of temperatures generally found on earth. In liquid form it is an excellent solvent for thousands of other substances, both organic and inorganic. Its liquid nature and great solvent power make it unexcelled as a transport medium. Blood, which is 90% water, serves to transport materials throughout our body. The fact that water dissolves so many substances means that these substances can be brought together in the form of individual molecules and ions. In this form, reactions between different substances can occur quickly. Water thus serves as the medium in which almost every chemical reaction in living things takes place. Water is also a reactant and/or product of many chemical reactions in living organisms. (Can you name one?)

Water is an excellent solvent

Another important property of water is the slowness with which it changes temperature or changes from solid (ice) to liquid, or liquid to gas, as heat is added. It takes more heat to accomplish these changes with water than with almost any other substance known. (Our unit of heat, the calorie, is, in fact, defined as the quantity of heat needed to raise one gram of water one degree Celsius—a table describing the metric system of measurement appears in the appendix.) This property of water is extremely important to life. It means that the temperature of living organisms changes relatively slowly despite sudden temperature changes in the environment. The great amount of heat absorbed when water vaporizes is exploited in sweating. Evaporation of water from the skin absorbs heat from the body, thus cooling it. Not only do the thermal properties of water affect organisms directly, but they also bring about a moderating effect in the environment. Freshwater and marine organisms are subject to a far smaller range of temperatures than land-living animals of the same region. Even land-living animals benefit from the temperature-moderating effect of nearby bodies of water. The ocean's slow absorption of heat in the summer and slow release of heat in the winter tend to reduce violent extremes of temperature in coastal regions. It is no accident that record low temperatures as well as record high temperatures are made in inland areas such as central Siberia, and central North America.

The heat capacity of water

Even when water does get cold enough to freeze, the ice that is formed is less dense than the water, and thus floats on top of the water. This enables aquatic organisms to remain surrounded by water under the ice and also provides for speedy melting of the ice in the spring. The solid form

of most substances is more dense than the liquid. It is interesting to speculate on what life in the "temperate" regions of the earth would be like if water were not an unusual substance in this respect.

In this chapter we have examined some of the chemical substances of which living things are composed. In the next chapter, we shall try to learn how these chemical substances are organized to make up the structures of the basic unit of life itself, the cell.

EXERCISES AND PROBLEMS

1. Pound for pound, what food yields the most metabolic water when oxidized?
2. What element is found in all proteins but in no oils?
3. Summarize the importance of water to living things.
4. What is a vitamin?
5. How does RNA differ from DNA?
6. Write the structural formula for the neutral fat tripalmitin (palmitic acid = $C_{15}H_{31}COOH$).
7. Write the structural formula for the tripeptide containing glycine—alanine—phenylalanine.
8. Show by means of structural formulas the hydrolysis of the tripeptide described in Question 7.
9. Distinguish between saturated and unsaturated fats.
10. Name an isomer of glucose; of sucrose.

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5 THE CELLULAR BASIS OF LIFE

The Cell "Theory"

Sizes and Shapes

The Cell Membrane

The Nucleus / The Cytoplasm

Ribosomes / The Endoplasmic Reticulum

The Golgi Apparatus

The Mitochondria

Plastids / Vacuoles / Lysosomes / Peroxisomes

Crystals and Oil Droplets

Microfilaments / Microtubules

Centrioles

Cilia and Flagella

Cell Coatings

Cell-to-Cell Junctions

The Cell as the Unit of Structure of Living Things

Plant Tissues / Animal Tissues

Higher Levels of Organization

The carbohydrate, lipid, protein, nucleic acid, and other molecules that make up living things are not themselves alive. It is only when these molecules become organized in precise ways that the phenomenon of life appears. The minimum organization of this matter that can live is called the cell.



Fig. 5.1 Robert Hooke's drawings of the cellular structure of cork were published in 1665. (Courtesy Bettmann Archive.)

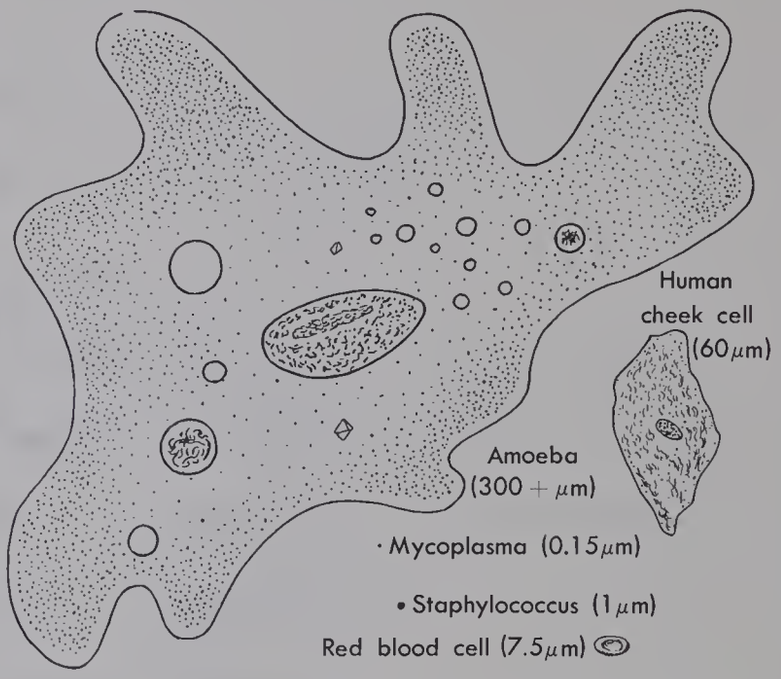


Fig. 5.2 Comparative sizes of various cells. The amoeba is just visible to the unaided eye. Mycoplasmas can be seen clearly only under the electron microscope.

5.1 THE CELL "THEORY"

We owe the term *cell* to the research of the Englishman, Robert Hooke. In 1665, he told of examining thin slivers of cork under his microscope and finding them to be made up of many neatly arranged little chambers (Fig. 5.1). He called these chambers *cells.* All that Hooke saw, however, were the cellulose walls laid down by once-living material. Today we use the term *cell* to include the active, living contents of such chambers. Sometimes the term *protoplast* is used when one wishes to emphasize that the active materials within any outer covering or wall are being considered.

"Protoplasm"

The term *protoplast* is itself derived from the word *protoplasm.* Protoplasm is the material that makes up the protoplast. The term is falling into disuse, however, because no single material *does* make up the protoplast. As we have seen, living material is composed of a large variety of different molecules. There is no single living substance. Actually, the word can still be useful to the biologist, but only as a shorter equivalent of "living material" or "the molecules that make up living things." In this sense, one

can speak of growth resulting in an increase in the amount of protoplasm. On the other hand, one should not use the term as though it referred to a single, specific substance. To say that "living things are made of protoplasm" in the sense that one says "sand is made of silica (SiO_2)" is as meaningless as to say that "television sets are made of videoplasm."

Schleiden and Schwann

The idea that the cell is the fundamental unit of which all living things are constructed grew out of the work of the German botanist Schleiden and the German zoologist Schwann. Schleiden trained his microscope on a wide variety of plant structures and in every case found that they were made of cells. Schwann made the same discovery in his microscopic examination of animal parts. Their dual discovery, which was set forth in 1839, is called the cell theory. It is not really a theory, though, but an observable fact of nature.

5.2 SIZES AND SHAPES

Cells occur in a variety of sizes and shapes. Eggs are single cells and most of them can be seen easily with the unaided eye. This is because of the bulky food reserves that they contain. Some of the larger microorganisms are just visible to the naked eye. An amoeba cell, some 300 μm across, is about the size of the periods printed on this page. (One micrometer, μm, is 10^{-6} meter or 0.001 millimeter. The micrometer is also commonly called a micron, μ. A table describing the metric system appears in the appendix.) Tissue cells are generally microscopic. In man, they may be as small as the red blood cells, which are 7.5 μm in diameter, or as large as the cells lining the inner surface of the cheek, which are some ten times that size. The smallest bacteria are just under 1 μm in diameter, while the smallest microorganisms known, the mycoplasmas, are only a tenth of that. The mycoplasmas probably represent the smallest size a cell can be and still have all the attributes of life (Fig. 5.2).

Most cells are too small to be seen by the unaided eye

Cells vary markedly in shape as well as size. The shape of a cell is usually a clear reflection of the function it carries out in the organism. Nerve cells, which must transmit electrical impulses over long distances, have long extensions, in some cases several feet in length. Muscle cells are elongated so that the force of contraction can be exerted efficiently in one direction. The supporting cells of a plant have thick walls. Where masses of cells are simply packed together, they assume the shape of a polyhedron, a solid with many faces. This shape permits close packing of the cells without a large amount of space between them.

5.3 THE CELL MEMBRANE

The outer surface of all protoplasts is bounded by a very thin, elastic membrane. It is so thin (75–100 angstroms, A; one A = 10^{-4} μm) that its structure can be observed only under the electron microscope.

The cell membrane provides an important barrier between the interior of the cell and the exterior. Water and many other small molecules pass

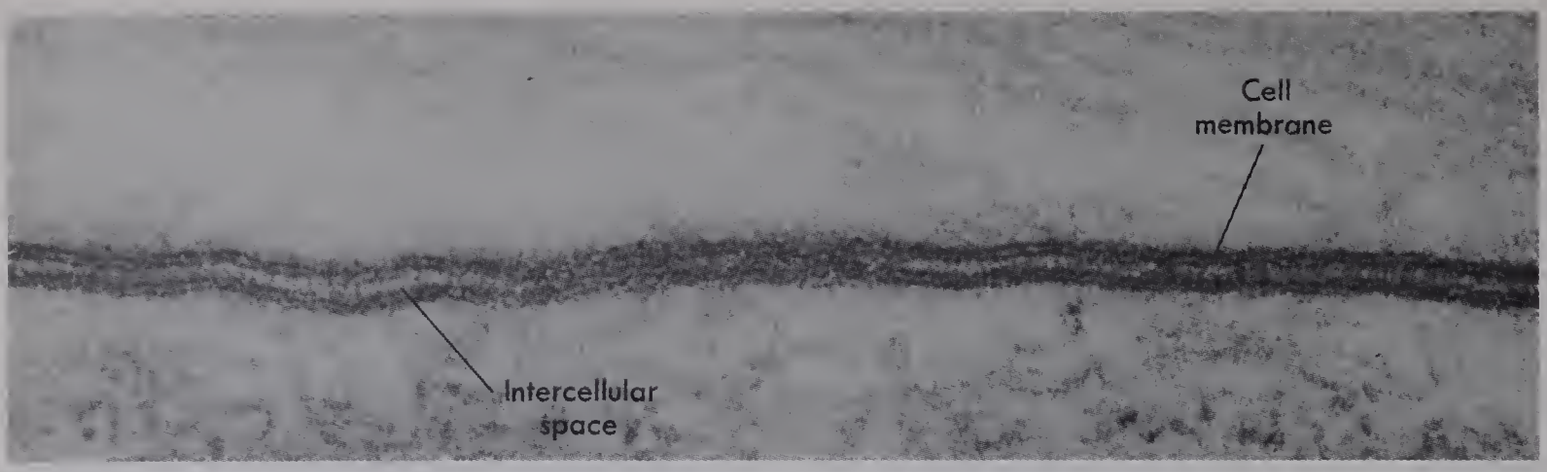


Fig. 5.3 Cell membranes (140,000 X). The membranes of *two* adjoining cells are shown here. The triple-layer construction of each of these "unit" membranes shows clearly. (From Fawcett: *The Cell: Its Organelles and Inclusions,* W. B. Saunders Co., 1966.)

The cell membrane regulates what enters and leaves the cell

through it quite easily. On the other hand, many ions such as the sodium ion (Na^+) and large molecules such as protein molecules pass through with greater difficulty. A membrane that permits the free passage of some materials and not others is called a semi- or differentially permeable membrane. The permeability of the cell membrane changes with changing conditions and this suggests that it is no passive barrier but exerts a selective action on which materials pass through it.

Chemical and physical studies of the cell membrane suggest that it is made up of three layers, of roughly equal thickness, sandwiched together. The outer layer seems to be composed chiefly of protein molecules although carbohydrates are also present. The middle layer is actually a double layer of phospholipid molecules. The inner layer is another protein layer. There is considerable evidence that some of the protein of both the outer and inner layers protrudes into the middle layer. The protein layers account for the elasticity of the membrane, since the folded protein molecules that make them up can be stretched a moderate amount. The lipid layer accounts for the ease with which many fat-soluble molecules pass through the cell membrane.

The picture of the cell membrane that has been derived from chemical and physical studies has been nicely supported by electron micrographs. Figure 5.3 shows the membranes of two adjacent cells. The sandwich construction of the membranes is clearly visible.

The different membranes of the cell represent variations on the same basic structural theme

Membranes of this construction bound the cells of all organisms: microorganisms, plants, and animals. Membranes of similar, often identical, construction are also found within the interior of cells. Here they serve to establish a variety of special compartments. In fact, the protein-lipid-protein membrane seems to be such a fundamental structural unit throughout the cell that it has been given a special name: the **unit membrane.** The use of this term should not suggest that all membranes in the cell have *precisely* the same chemical composition and physical organiza-

tion. Different membranes serve different functions and these differences are reflected in their chemical and physical properties. The amount and types of proteins present, for example, may vary greatly between two types of membranes.

Except in the bacteria and blue-green algae, there are two major compartments of the cell, the cytoplasm and the nucleus.

5.4 THE NUCLEUS

The nucleus is separated from the cytoplasm by a pair of unit membranes. The envelope thus formed is not continuous but, as can be seen in Fig. 5.4, contains pores. These probably permit materials to pass between the nucleus and the cytoplasm.

Within the nuclear membrane there is a semifluid medium in which are suspended the **chromosomes.** Usually these are present as very elongated structures and cannot be easily observed under the light microscope. The term *chromatin* is used to describe them when they are in this condition.

Chromatin

When a cell is preparing to divide into two cells, the appearance of the chromosomes changes. The long thin strands coil up into thickened, dense bodies, which (with the help of an appropriate stain) are easily visible in the light microscope (Fig. 5.17). During the process of cell division, the chromosomes are distributed in precisely equal numbers to the two daughter cells.

The chromosomes of most cells can be seen only at the time of cell division

Chemically, the chromosomes are made up of DNA and protein. We do not yet know whether a single chromosome contains only a single DNA molecule or many of them. The protein is a special type called *histone.*

During the period between cell divisions, when the chromosomes are in the extended state, one or more of them may have a large, spherical body attached. This body, the **nucleolus,** is easily visible in the light microscope. Here are synthesized several kinds of RNA molecules. Some of this RNA is used in the assembly of **ribosomes.** Ribosomes are essential for protein synthesis in the cell, so it is not surprising that cells that are very active in protein synthesis usually have extra-large nucleoli.

Function of the nucleolus

The nucleus functions as the control center of the cell. Our appreciation of its role has come from many experiments and observations. In particular, the development of micromanipulators has enabled cytologists to remove or transplant nuclei from single cells and to study the results of these operations. If the nucleus is removed from an amoeba, the organism continues to live for a few days. It cannot reproduce, however, and eventually it dies. That it is the loss of the nucleus which causes this and not just mechanical damage from the operation can be shown by piercing an amoeba with the microtools but not actually removing the nucleus. Such an amoeba recovers fully from this experimental procedure.

Microsurgery can be carried out on individual cells

Even before the invention of micromanipulators, the importance of the nucleus in determining what the cytoplasm does had been demonstrated by the German biologist Theodor Boveri. By vigorous shaking,

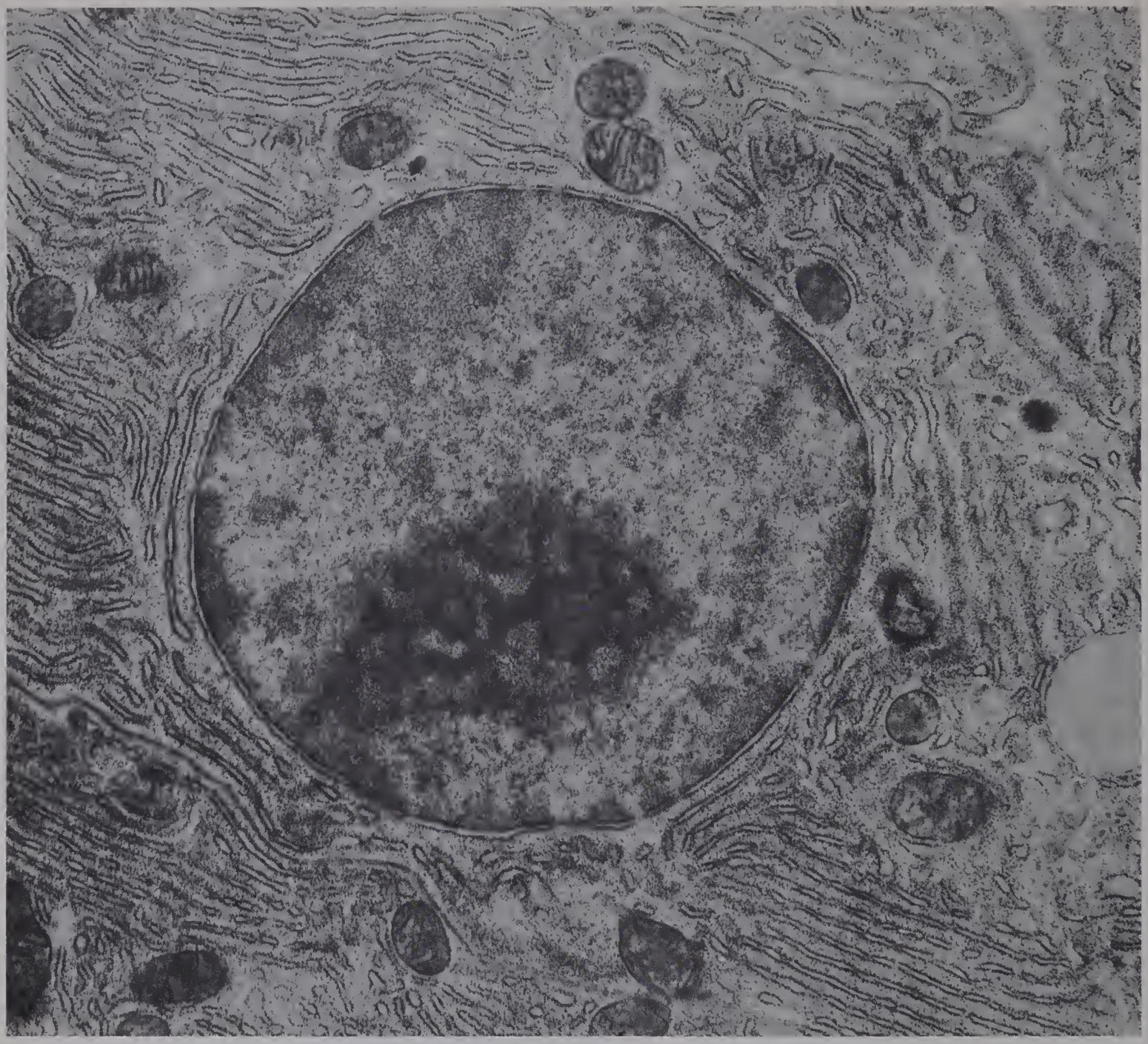


Fig. 5.4 The nucleus and surrounding cytoplasm of a cell from the pancreas of a bat (18,000 X). Note the double-layer construction of the nuclear membrane and the pores in it. What other cell structures can you identify? (Electron micrograph courtesy of Dr. Don W. Fawcett.)

Boveri's experiment

Boveri was able to remove the nucleus from the eggs of a species of sea urchin of the genus *Sphaerechinus.* He then allowed these "enucleated" eggs to be fertilized by sperm from sea urchins of the genus *Echinus.* Sperm cells are much smaller than egg cells. They consist of little more than a nucleus and a tail to propel it. In the process of fertilization, the nucleus penetrates the egg. Thus fertilization of enucleated *Sphaerechinus* eggs by *Echinus* sperm resulted in the substitution of one kind of nucleus for

another. The stimulus of fertilization caused the egg to undergo cell division and it developed into a sea urchin larva. A glance at Fig. 5.5 shows that this larva possessed the traits of the *Echinus* species rather than of the *Sphaerechinus* species. Although *Echinus* contributed little more than a single tiny nucleus to the system, this nucleus caused the great mass of *Sphaerechinus* cytoplasm to develop according to the *Echinus* blueprint.

5.5 THE CYTOPLASM

The term *cytoplasm* suffers from much the same defect as the term *protoplasm.* Cytoplasm is a complex, heterogeneous material. Basically, it consists of a rather watery medium, the "ground substance," in which is suspended a variety of distinct structures. The functions of the cytoplasm are, for all practical purposes, the functions of the structures suspended in it. We shall now examine these.

5.6 RIBOSOMES

Ribosomes assemble amino acids into proteins

Among the smallest structures suspended in the cytoplasm are the ribosomes. These roughly-spherical bodies are so small (150 A) that they can be seen only under the electron microscope (Fig. 5.4). They are composed of RNA and protein. All the proteins of the cell, including all enzymes, are synthesized by the ribosomes. As we shall see in Chapter 19, the ribosomes literally carry out the instructions contained within the DNA code of the nucleus.

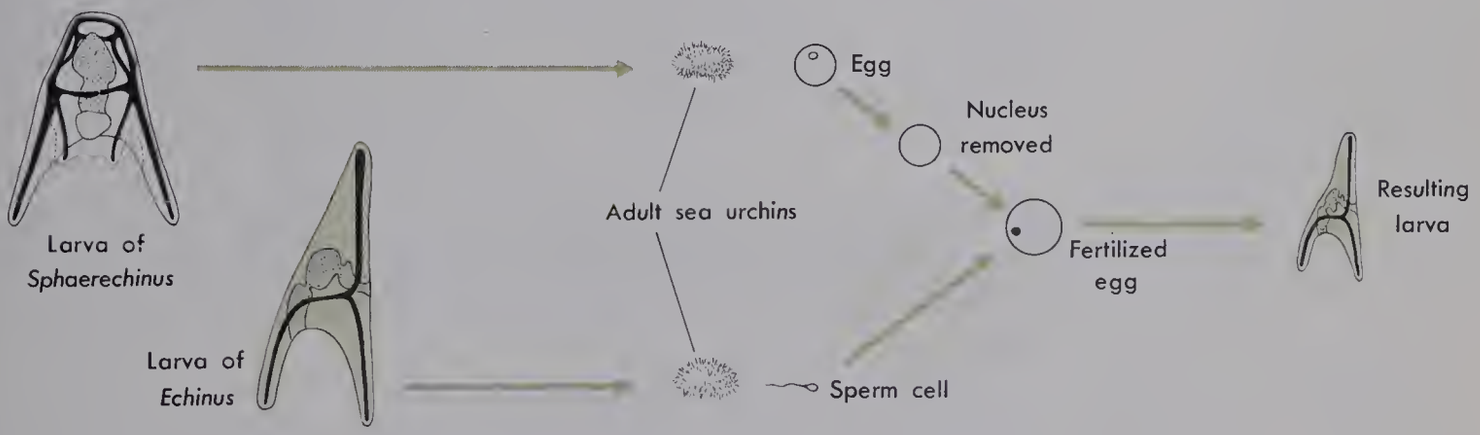


Fig. 5.5 Boveri's experiment, which demonstrated the importance of the nucleus in controlling the cell. An *Echinus* nucleus in *Sphaerechinus* cytoplasm resulted in a larva with *Echinus* characteristics.

The ribosomes that are engaged in synthesizing proteins for use within the cell are distributed randomly in the ground substance of the cell. However, many cells, such as those of the liver and pancreas, also synthesize proteins that are secreted *outside* the cell. The ribosomes that synthesize these proteins are found attached to the membranes of the endoplasmic reticulum.

5.7 THE ENDOPLASMIC RETICULUM

Substances destined to be secreted from the cell are synthesized in the endoplasmic reticulum

The endoplasmic reticulum is an elaborate system of membranes that have the same basic structure as the other membranes we have described. They are organized in pairs and thus form totally enclosed spaces, or vesicles, within the ground substance of the cell. If you look closely at Fig. 5.4, you can see rows of ribosomes adhering to the outer surface of the reticular membranes, that is, the surface in contact with the ground substance of the cell. The protein that is produced by these ribosomes is secreted into the cavity of the endoplasmic reticulum for eventual export to the outside.

Not all the membranes of the endoplasmic reticulum have ribosomes adhering to them. Those that do not (the "smooth" endoplasmic reticulum) may be active in the synthesis and/or accumulation of other types of molecules such as polysaccharides and steroids.

5.8 THE GOLGI APPARATUS

Functions of the Golgi apparatus

The Golgi apparatus consists of a stacked array of membrane-bounded vesicles. It is particularly prominent in cells that are active in secretion. There is good evidence that materials synthesized within the endoplasmic reticulum are transferred to the Golgi apparatus, where they are assembled into granules prior to their export from the cell. The Golgi apparatus is not simply a passive packaging device, however. Many proteins secreted by cells have carbohydrates attached to them, and some of this synthesis occurs in the Golgi apparatus. The Golgi apparatus also plays a role in the synthesis of complex polysaccharides, such as mucus and components of the cell wall of certain algae. There is also evidence that the Golgi apparatus manufactures lysosomes and additional cell membrane (to replace that used up in the formation of vacuoles).

5.9 THE MITOCHONDRIA

The mitochondria are spherical or rod-shaped bodies that range in size from 0.2 μm to 5 μm. The number in a cell varies, but active cells (e.g. liver cells) may have over a thousand of them.

The structure of mitochondria

Although the larger mitochondria can be seen under the light microscope, only the electron microscope can reveal their basic structure. Electron micrographs show that each mitochondrion is bounded by a double membrane. The outer membrane provides a smooth, uninterrupted boundary to the mitochondrion. The inner membrane is repeatedly extended into folds that project into the interior space of the mitochondrion (Fig. 5.6). These shelflike inner folds are called **cristae.** Adhering to their surface are a very large number of different kinds of enzyme molecules.

The membranes of the mitochondria appear to be similar to the cell membrane. Like it, they consist of a double layer of phospholipid molecules sandwiched between layers of protein molecules. Also like cell membranes, they can expand and contract. In fact, mitochondria are frequently observed to enlarge or shrink as the metabolic activity of the cell varies.

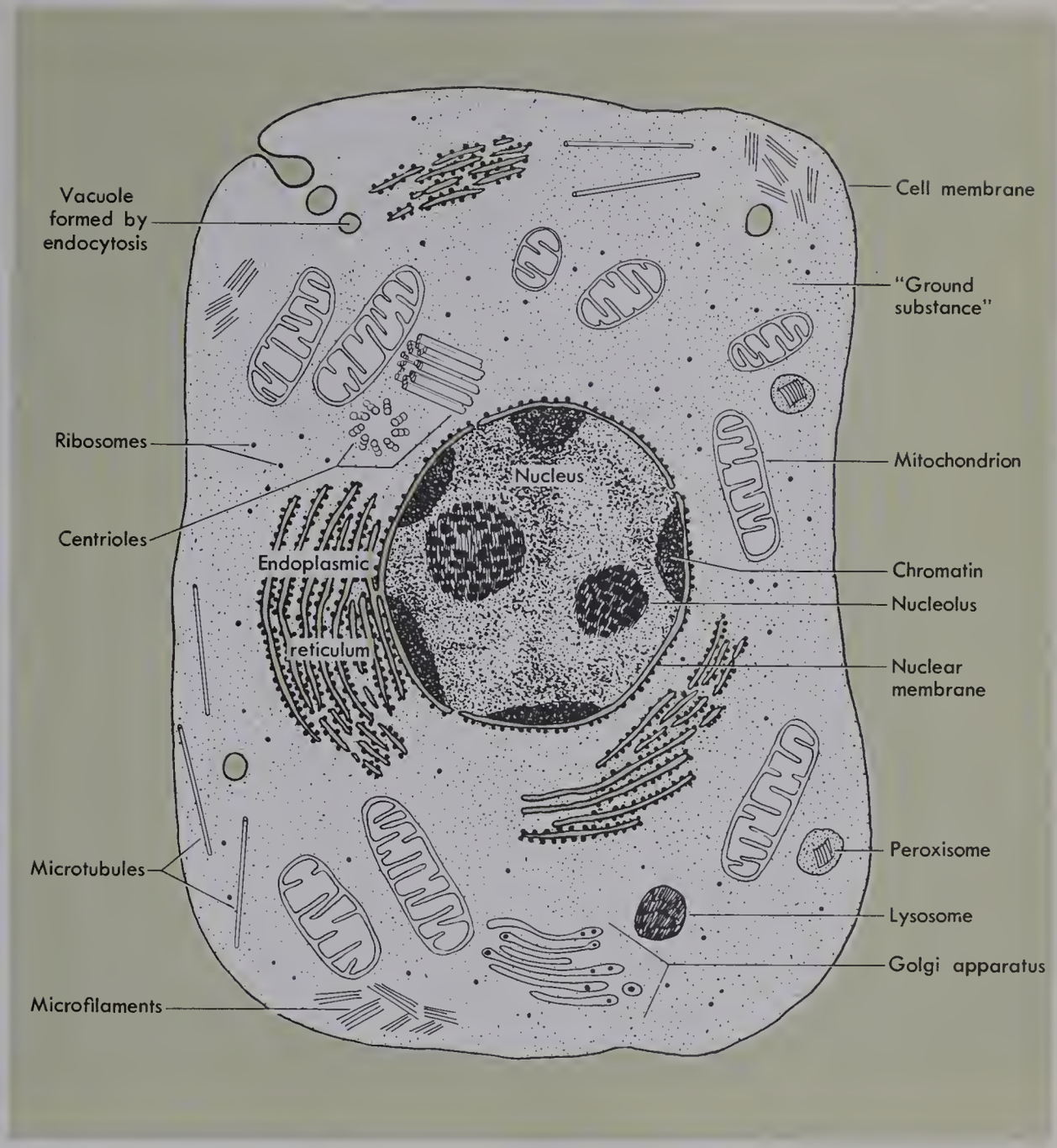


Fig. 5.6 An idealized view of an animal cell as it might be seen under the electron microscope. Although no single electron micrograph has revealed all the structures shown, the drawing represents a composite view of what many electron micrographs suggest is the organization of the parts of the cell.

Mitochondria generate energy for use within the cell

The function of the mitochondria is quite clear. They contain the enzymes that carry out the oxidation of food substances. Thus the mitochondria convert the potential energy of different food materials into a form of energy that can be used by the cell to carry out its various activities. In view of this, it is not surprising that mitochondria tend to congregate in the most active regions of a cell. Nerve cells, muscle cells, and secretory cells all contain many mitochondria located in the regions of the

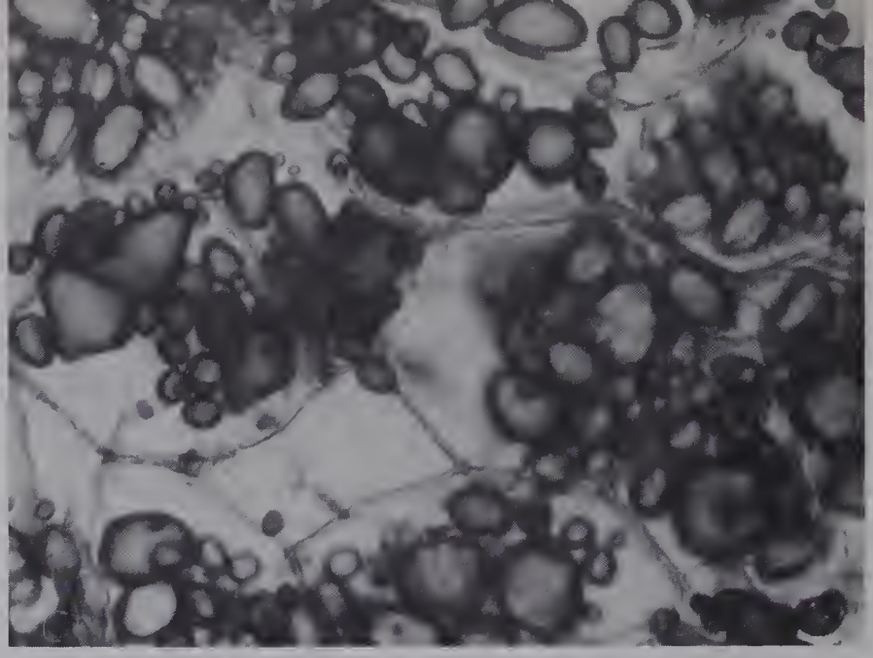


Fig. 5.7 Starch grains in the cells of a white potato. Note the cell walls. The starch grains have been lightly stained with iodine.

cell most actively engaged in transmission of electrical impulses, contraction, and secretion, respectively. The mitochondria have been aptly called the powerhouses of the cell.

5.10 PLASTIDS

Plastids are found only in plant cells and the cells of most photosynthetic microorganisms. Some plastids contain red, yellow, or orange pigments and impart these colors to flowers and ripe fruits. Some plastids are colorless. These are usually found in plant parts that are not exposed to the light and serve as a storage area for starch. The starch accumulates in grains. Figure 5.7 shows starch grains in the cells of the white potato.

Chloroplasts manufacture food from inorganic raw materials

By far the most important plastids are the chloroplasts. These contain the green pigment **chlorophyll.** It is chlorophyll which traps the energy of sunlight and enables it to be used for the manufacture of food. Thus the chloroplasts are the site at which photosynthesis takes place.

In plants, the chloroplasts are usually disk-shaped structures, 5–8 μm in diameter and 1 μm thick. Photosynthesizing cells may contain as many as 50 or more of them. Among the algae, the chloroplasts may assume a wide variety of shapes and only one or a few be present in a cell. Figure 5.16 shows the single cup-shaped chloroplast of the microscopic green alga *Chlamydomonas.*

Chemical studies show that chloroplasts are rich in structural protein and phospholipids. This suggests that a protein-lipid-protein unit membrane may also be present in chloroplasts, and electron micrographs have indeed revealed its presence. Under the electron microscope, the chloroplast is seen to be bounded by a unit membrane and also to contain interconnecting stacks of membranes in its interior (Fig. 5.8). These, in turn, appear to be made up of layers of chlorophyll and lipid molecules sandwiched between the layers of protein. This arrangement spreads the chlorophyll molecules over a large area and seems to provide for maximum efficiency of light absorption and photosynthesis.



Fig. 5.8 Cells from the leaf of a sunflower. Note the nucleus (N), chloroplasts (Chl), mitochondria (M), crystals (Cry), central vacuole, and primary cell wall in these typical plant cells. (Electron micrograph courtesy of H. J. Arnott and Kenneth M. Smith).

5.11 VACUOLES

Vacuoles are fluid-filled "bubbles" in the cytoplasm. They are bordered by a unit membrane that is probably identical to the cell membrane. In fact, vacuoles are often formed by an infolding and pinching-off of a portion of the cell membrane (Fig. 5.6). Food materials or wastes may be found inside vacuoles.

A young plant cell contains many small vacuoles, but as the cell matures, these unite to form a large **central vacuole** (Fig. 5.8). Dissolved food molecules, waste materials, and pigments may be found in it.

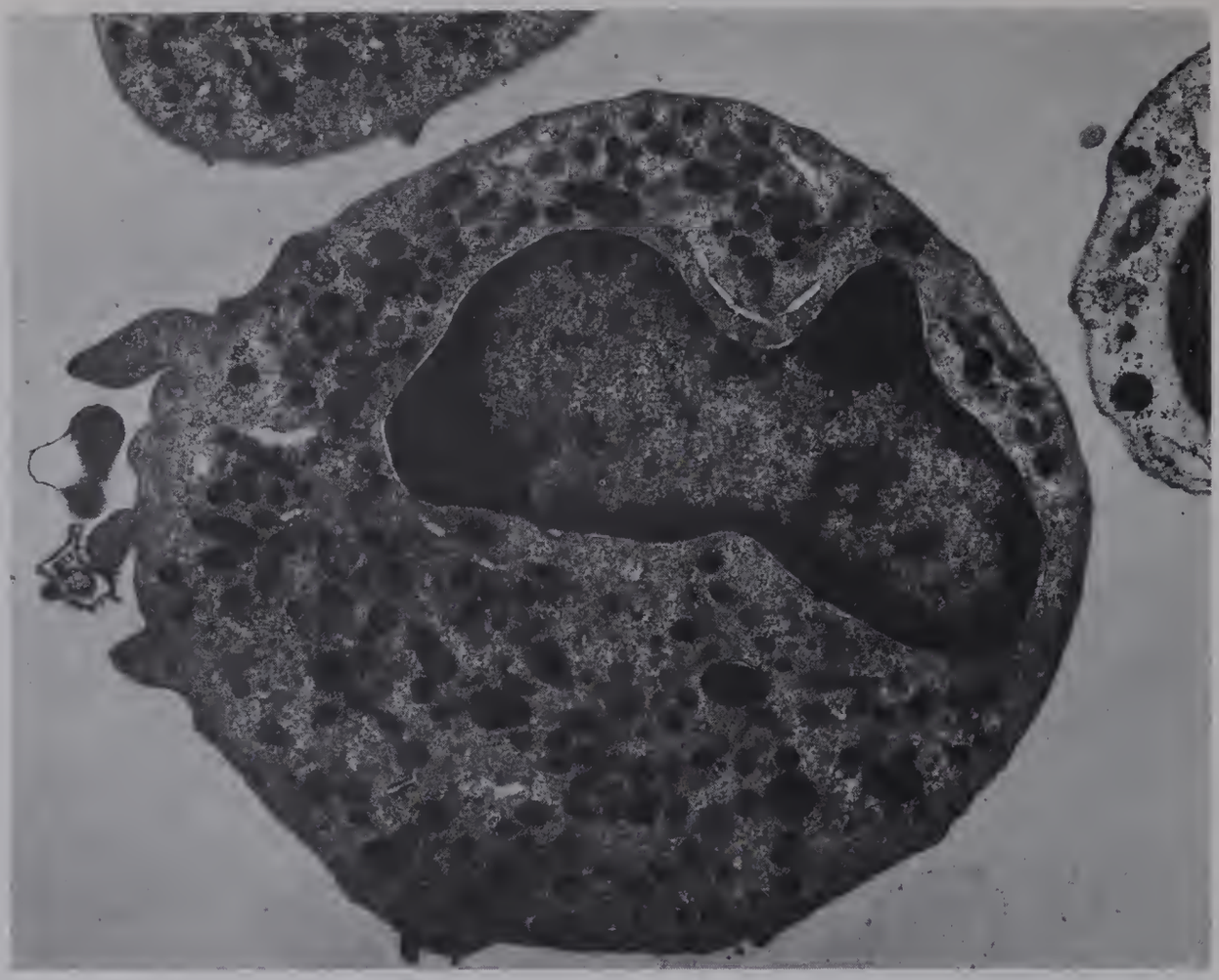


Fig. 5.9 White blood cell from a guinea pig. The small dark bodies in the cytoplasm are lysosomes. (9500 X, from Fawcett: *The Cell: Its Organelles and Inclusions,* W. B. Saunders Co., 1966.)

5.12 LYSOSOMES

Lysosomes (Fig. 5.9) are cell structures about the size of mitochondria and bounded by a unit membrane. Within them are contained a number of enzymes that catalyze the breakdown of polysaccharide, protein, and nucleic acid molecules. Presumably, these enzymes are kept within the lysosomes to prevent breakdown of the cell itself.

Lysosomes contain digestive enzymes for use within the cell

When materials within the cell are to be digested, they are first incorporated into a lysosome. These materials may be other subcellular structures, such as mitochondria, that have ceased to function efficiently. They may be food particles that have been taken into the cell. In the case of the white blood cell shown in Fig. 5.9, they are bacteria or other harmful particles that have been scavenged by the cell.

Lysosomes also play an important role in the death of cells. When a cell is injured or dies, its lysosomes aid in its disintegration. This clears the area so that a healthy cell can replace the damaged one. Cell death is also a normal process in some organisms. For example, as a tadpole changes into

a frog, its tail is gradually absorbed. The tail cells, which are richly supplied with lysosomes, die and the products of their disintegration are used in the growth of new cells in the developing frog.

Lysosomes have not been conclusively shown to occur in plant cells.

5.13 PEROXISOMES

Many cells, both plant and animal, contain membrane-bounded structures that somewhat resemble lysosomes. Like lysosomes, they are filled with enzymes of which catalase (see Section 3.11) is the most characteristic. Peroxisomes may play an important role in converting fats into carbohydrates. A number of other metabolic functions have been assigned to peroxisomes in particular tissues or organisms, but except for the activities of catalase, no single function is common to *all* peroxisomes. Peroxisomes are probably manufactured by the endoplasmic reticulum.

5.14 CRYSTALS AND OIL DROPLETS

Crystals are sequestered within a membrane

Crystals are deposits of food or waste materials that are surrounded by a unit membrane. As the name implies, the deposits are crystalline, that is, polyhedrons with flat faces and sharp edges. Crystals are most common in plant cells (Fig. 5.8) and are usually deposits of calcium salts. These are presumably waste products of the cell's metabolism. In animal cells, the crystals are generally protein.

Oil droplets are common in both plant and animal cells. They differ from crystals in that there is usually no unit membrane separating the oil from the rest of the cytoplasm. Under the microscope, the droplets generally appear as tiny, glistening spheres. However, in the specialized fat storage cells of some plant and animal tissues, the amount of oil present may be so great as to practically fill the entire cell. Oil droplets serve as a reserve supply of energy-rich fuel for the cell.

Membranes establish functional compartments within the cell

Most of the cell structures discussed so far have unit membranes as part of their construction. In general, there seem to be two main functions accomplished by these membranes. One is simply to establish a number of compartments within the cell. The bearers of the hereditary code, the chromosomes, are separated from the rest of the cell by the nuclear membrane. The potentially destructive digestive enzymes in the lysosomes are kept from contact with the ground substance of the cytoplasm by their bounding membrane. The secretory products of the cell are sequestered in the channels of the endoplasmic reticulum and Golgi complex.

The second important role played by the membranes of the cell is to provide for the neat spatial organization of enzymes and pigments. Chlorophyll is incorporated in the internal membranes of the chloroplast. The enzymes which carry out the oxidation of food are neatly arranged on the cristae of the mitochondria. It is quite likely that other important enzymes are present in or on the cell membrane and the membranes of the endoplasmic reticulum.

5.15 MICROFILAMENTS

Microfilaments are long, thin protein fibers some 40–60 A in diameter. Large numbers of them form masses or networks at various places in the cell (Fig. 5.10). Their presence is associated with cell movement. When an animal cell divides in two, for example, a band of microfilaments forms and pinches the two daughter cells apart.

Microfilaments are necessary for several kinds of cell movement

The cytoplasm moves about in many cells—a phenomenon known as cytoplasmic streaming. Its motion is dependent on the presence of microfilaments. Microfilaments are also prominent features in cells that migrate from place to place and change their shape. Not only is this true for an independent, free-moving cell such as an amoeba but also for most animal cells during the formation of an embryo. The pinching-off of infolded portions of the cell membrane (see Section 5.11) also depends on the contractility provided by microfilaments.

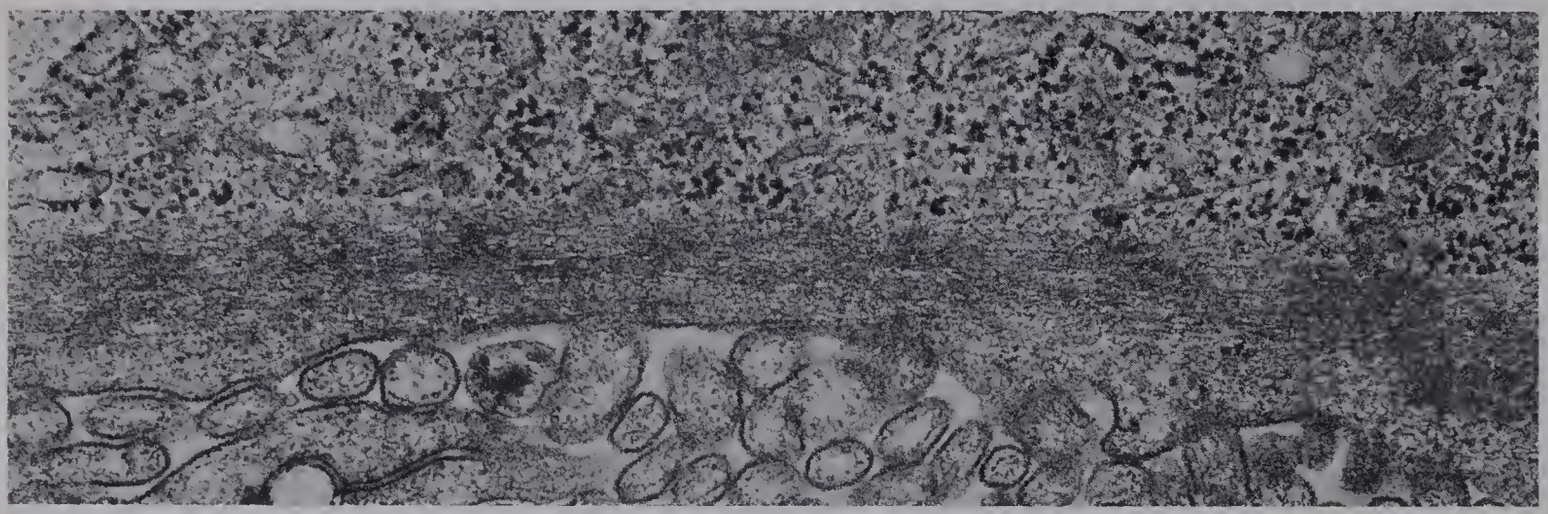


Fig. 5.10 A band of microfilaments (running horizontally) in a cell of the chick oviduct. The dark dots scattered above the microfilaments are ribosomes. (Electron micrograph courtesy of J. T. Wrenn.)

5.16 MICROTUBULES

Microtubules are straight cylinders of protein that are found in many cells. These cylinders are about 250 A in diameter and quite long (Fig. 5.6). They are also quite stiff and therefore provide some rigidity to those parts of the cell in which they are located. They are especially apt to be found near the margin of the cell, where they appear to act as an intracellular skeleton.

Microtubules impart rigidity to the contents of the cell

Microtubules also play an essential role in cell division. Successful cell division requires a precise distribution of chromosomes to each daughter cell. Each chromosome moves to its final destination attached to a long microtubule. The entire array of microtubules participating in the process is called the spindle. It can be seen in several of the photomicrographs of dividing cells that appear in Chapter 16. Microtubules are also used in the construction of centrioles, cilia, and flagella.

5.17 CENTRIOLES

Centrioles are constructed from microtubules

Animal cells and the cells of some microorganisms and lower plants contain two centrioles located near the exterior surface of the nucleus. Each centriole consists of a cylindrical array of nine microtubules. However, each of the nine microtubules has two partial (as seen in cross section) tubules attached to it (Fig. 5.6). The two centrioles are usually placed at right angles to each other.

Just before a cell divides, its centrioles duplicate and one pair migrates to the opposite side of the nucleus. The spindle (see above) then forms between them.

In some cells, the centrioles duplicate to produce the basal bodies of cilia and flagella.

5.18 CILIA AND FLAGELLA

Many cells have whiplike extensions, either short ones (cilia) or long ones (flagella). In microorganisms such as *Chlamydomonas* (Fig. 5.16), cilia and flagella are used for locomotion. However, many animals have cells the cilia of which serve simply to move materials past the cell. The cells lining the inner surface of our trachea ("windpipe") are ciliated.

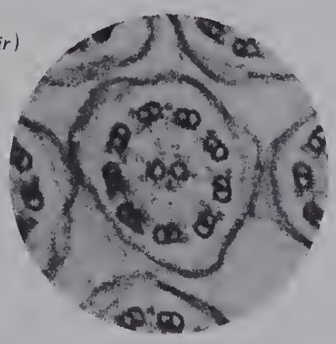
The origin and structure of cilia and flagella seem to be fundamentally the same. In each case they grow out of basal bodies. These have the same structure as centrioles and are formed by them.

The "9 + 2" arrangement

The cilium or flagellum itself has not only the outer ring of 9 microtubules (each now with just *one* accessory tubule attached to it) but also two central fibrils that are identical to microtubules in their construction (Fig. 5.11). In both cilia and flagella, the entire assembly is sheathed in a unit membrane which is simply an extension of the cell membrane.

The similarities between the structure of cilia, flagella, basal bodies, and centrioles and the structure of microtubules suggest that the microtubule is another one of the fundamental architectural components of cells. In function, also, it is interesting that in most cases where the microtubular structure appears, it is in some way associated with cell motion.

(*Courtesy Peter Satir*)



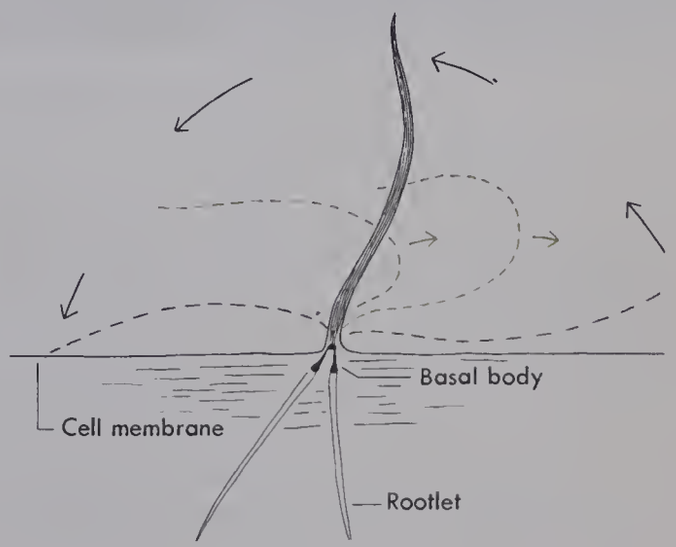


Fig. 5.11 At right, drawing of a single cilium. The power stroke is shown in black; the recovery stroke, in color. Above, an electron micrograph of a single cilium in cross section. Note the characteristic pattern of microtubules.



Fig. 5.12 Filaments of collagen, a protein, 31,000 X. (Courtesy of Dr. Jerome Gross.)

5.19 CELL COATINGS

Collagen is the chief extracellular protein of animals

Only rarely is the cell membrane the outer surface of the cell. Usually some type of exterior coating is present. In animal cells this appears to be constructed from a protein-polysaccharide complex. It is not rigid but does serve to cement adjacent cells together. In Fig. 5.3, it is represented by the thin line between the two unit membranes. In many cases, however, it is much thicker than shown here. Filaments of protein collagen (Fig. 5.12) are often embedded in the thicker coatings.



Fig. 5.13 Fibrils of cellulose in the cell wall of a green alga, 26,000 X. (Electron micrograph courtesy of R. D. Preston.)

The cell wall

In many algae and in all plants, the exterior coating is made of the polysaccharide cellulose. This forms a rigid, boxlike **cell wall** which is one of the most characteristic features of these cells. The "cells" that Hooke saw (Fig. 5.1) were the walls of once-living cork cells. In Fig. 5.8, the cell wall that surrounds each of the sunflower cells is clearly visible.

The cellulose molecules that make up the cell wall are deposited in an orderly pattern or "weave" that increases the mechanical strength of the wall (Fig. 5.13). As the cell grows, the wall grows, too, in a few cases as much as a thousandfold. The cell wall is porous, permitting molecules, both small and large, to pass through it with relative ease. Unlike the cell membrane, it does not exert any control over the kinds of molecules that pass through it.

Rigid cell walls are also characteristic of bacteria and fungi. However, polysaccharides other than cellulose are used in their construction.

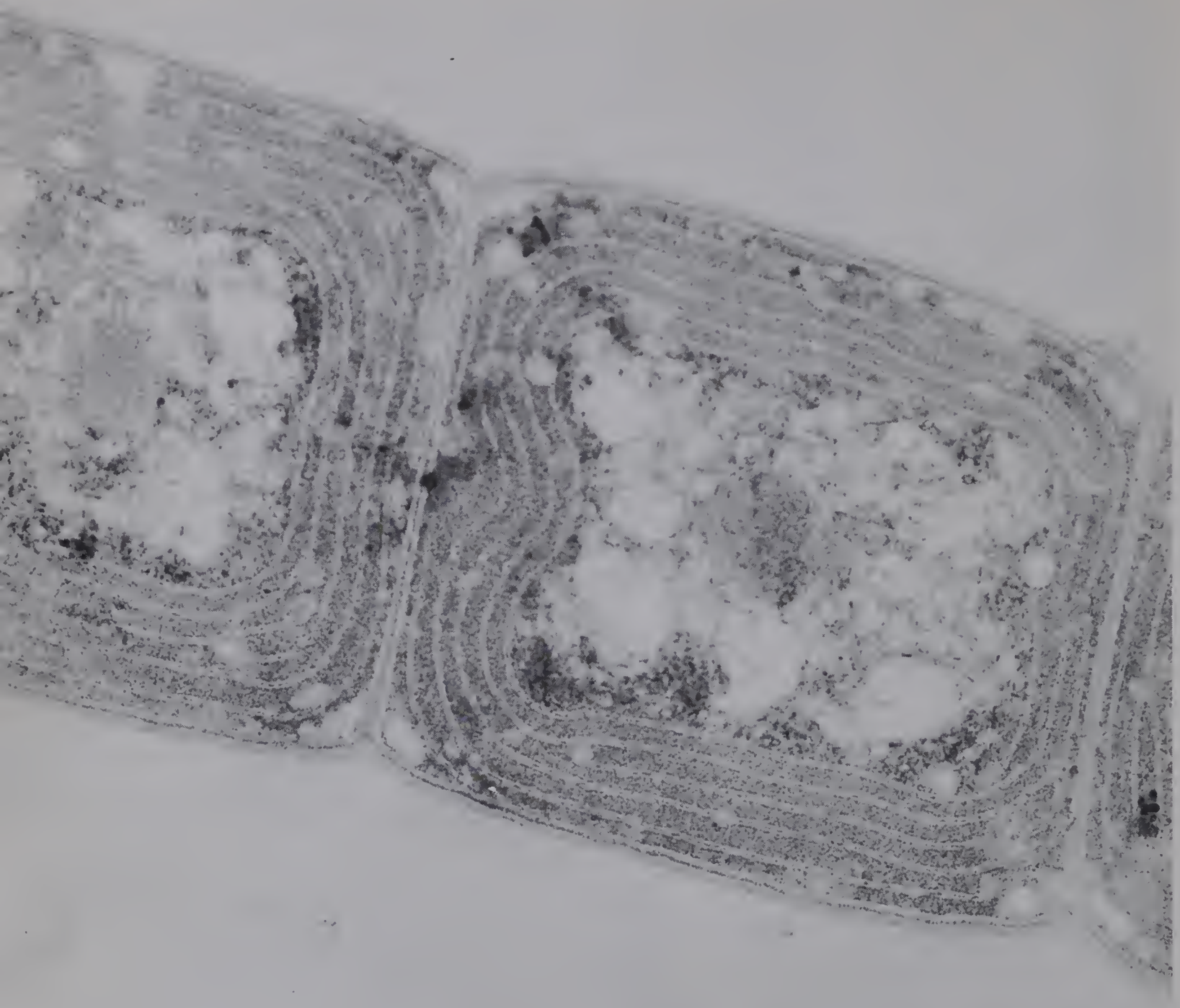
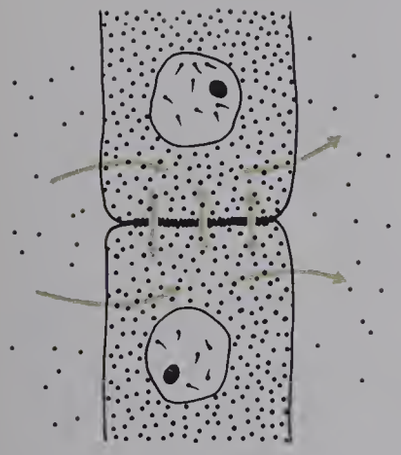


Fig. 5.14 Cells of the blue-green alga *Plectonema boryanum,* 45,000 X. The cells of this organism do not contain a membrane-bounded nucleus nor other membrane-bounded organelles such as mitochondria and plastids. Such cells are called prokaryotic ("pre-nuclear"). All blue-green algae and bacteria are prokaryotic. (Courtesy Kenneth M. Smith and R. Malcolm Brown, Jr.)

5.20 CELL-TO-CELL JUNCTIONS

In multicellular organisms, cells are packed together to form tissues. Generally, an extracellular coating separates the cell membrane of one cell from that of the adjacent cells in the tissue. However, in certain tissues (e.g. the lining of the intestine) the membranes of two adjacent cells are pressed directly together along a narrow band. This is known as a **tight junction.** It is probably of considerable physiological importance because it presents a barrier to the diffusion of molecules and ions through the space between cells. Where tight junctions are present, materials must actually enter and pass through cells in order to pass through the tissue. This pathway surely provides greater control over what substances are permitted through than would be the case if diffusion between the cells occurred.



Tight junctions may also serve as a pathway of intercellular communication. It has been known for some time that ions (hence electrical currents) and molecules (up to a molecular weight of about 10,000 daltons) pass readily from one cell to the next in certain tissues, but do *not* pass easily to and from these cells and the exterior. Presumably this special pathway between cells occurs at the tight junctions and provides a mechanism by which some sorts of signals (as yet unknown) can pass from cell to cell.

While not *all* cells contain *all* the structures discussed in this chapter, most of these structures are commonly found in the cells of animals, plants, and even in most microorganisms. However, two groups of microorganisms—the bacteria and blue-green algae—have cells that differ from the cells of other organisms in several notable respects. Their cells have no membrane-bounded nucleus, no mitochondria, plastids, endoplasmic reticulum, Golgi apparatus, or vacuoles (Fig. 5.14).

Prokaryotes vs. eukaryotes

The term *prokaryotic* is often used to differentiate the cells of these organisms from the nucleus-containing cells of all other (*eukaryotic*) organisms. While some bacteria have flagella, these consist of simply a single strand, like a single microtubule. There is not the multistranded, "9 + 2" arrangement that we find in the cilia and flagella of other organisms. The absence of so many structures from the cells of organisms in these two groups suggests that these creatures are quite primitive in their organization. Nevertheless, they are capable of carrying out all the essential functions of life.

5.21 THE CELL AS THE UNIT OF STRUCTURE OF LIVING THINGS

We have seen that living things are made of one or more cells. The microscopic green alga *Chlamydomonas* is a single-celled organism. Within its single cell, it contains all the equipment needed to carry out the various functions of life. From time to time, *Chlamydomonas* divides and forms two individuals where before there was one. Each daughter cell receives a complete set of the nuclear controls present in the parent cell. Prior to actual division of the cell, each chromosome in the nucleus is duplicated.

Mitosis is the separation of duplicated chromosomes

Then, during the process of cell division itself, these duplicated chromosomes become separated. With remarkable precision, a complete set migrates to each of the two daughter cells. **Mitosis** is the term used to describe this important process. It provides a mechanism for the reproduction of single-celled organisms. It also provides a mechanism for growth in multicellular organisms. In both cases, it provides an escape from excessive growth or enlargement of single cells. Small cells can usually operate more efficiently than large ones. One reason for this is that small cells have a larger ratio of surface area to volume than big ones (Fig. 5.15). Thus a more rapid exchange of materials can occur between the cytoplasm and the external environment of the cell.

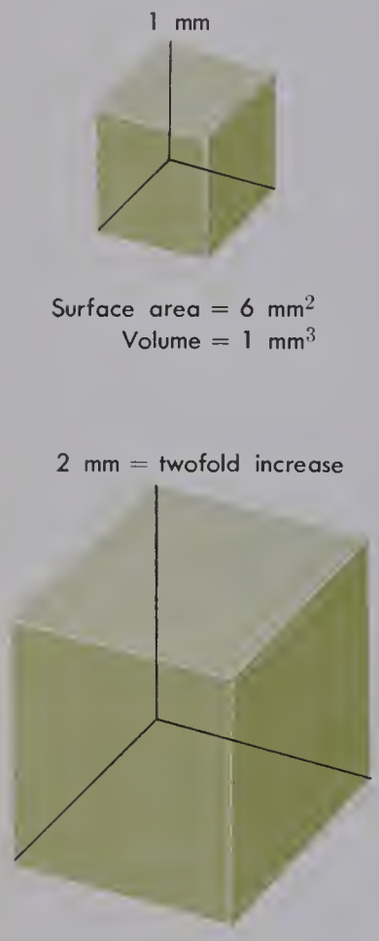


Fig. 5.15 The ratio of surface area to volume of the cube decreases as its size increases. The same relationship holds for cells and organisms.

Among the flagellated green algae are several interesting colonial forms (Fig. 5.16). These species are called colonial because they are simply made up of clusters of independent cells. If a single cell of *Gonium, Pandorina,* or *Eudorina* is isolated from the rest of the colony, it will swim away looking quite like a *Chlamydomonas* cell. Then, as it undergoes mitosis, it will form a new colony with the characteristic number of cells in that colony. The situation in *Pleodorina* and *Volvox* is somewhat different. In these cases, some of the cells of the colony (most, in *Volvox*) are not able to live independently. If a nonreproductive cell is isolated from a *Volvox* colony, it will fail to reproduce itself by mitosis and eventually it will die. What has happened? In some way, as yet unclear, *Volvox* has crossed the line separating simple colonial organisms from truly multicellular ones. Unlike *Gonium, Volvox* cannot be considered simply a colony or cluster of individual cells. It is a single organism whose cells have lost their ability to live independently. If a sufficient number of them become damaged for some reason, the entire sphere of cells will die.

What has *Volvox* gained by this arrangement? In giving up their independence, the cells of *Volvox* have become specialists. No longer does every cell carry out all of life's functions (as in colonial forms); instead, certain cells specialize to carry out certain functions while leaving other functions to other specialists. In *Volvox* this process goes no further than having certain cells specialized for reproduction while others, unable to reproduce themselves, fulfill the needs for photosynthesis and locomotion. In more complex multicellular organisms, such as man, the degree of specialization is carried to greater lengths. Each cell has one or two precise functions to carry out. It depends on other cells to carry out all the other functions necessary to maintain the life of the organism and thus its own. This process of specialization and division of labor among cells is called **differentiation.** One of the great problems of biology is how differentiation arises among cells, all of which, having arisen by mitosis, share the same nuclear controls. We shall examine some tentative answers to this problem in Chapter 22.

Specialization and division of labor results in increased efficiency in the same way that a modern shoe factory is more efficient than the individual bootmaker of earlier times. A price must be paid, however, for this increased efficiency. If accurate communication and coordination between

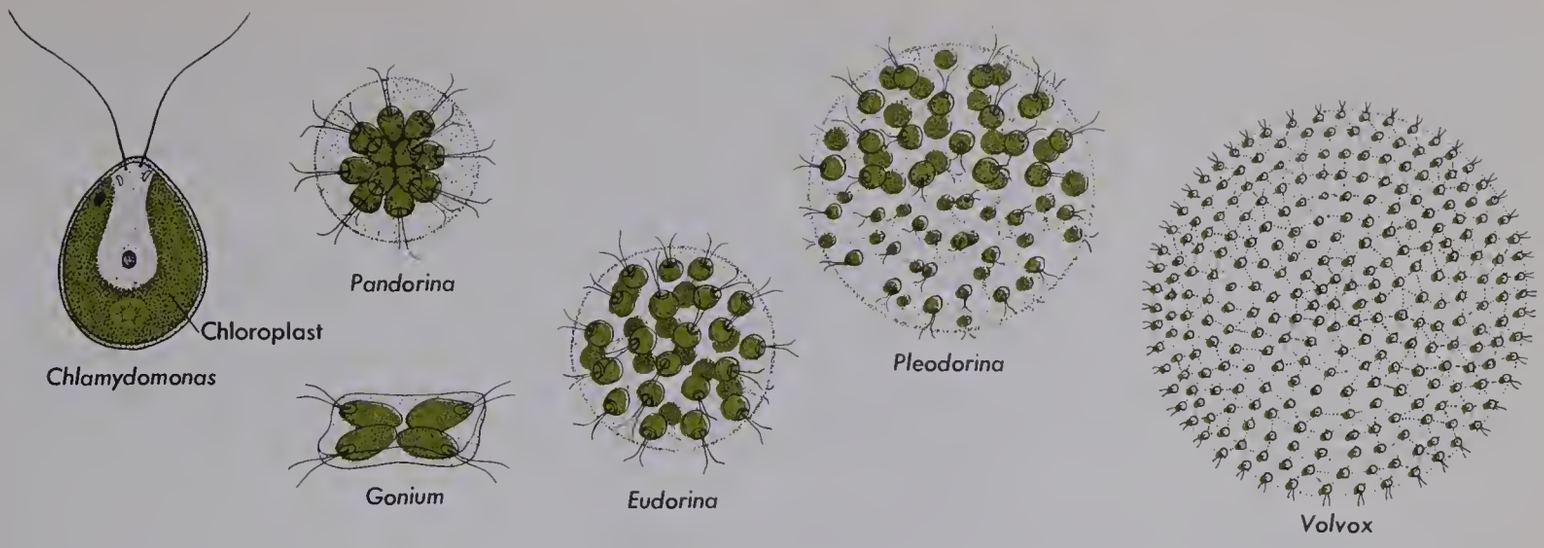


Fig. 5.16 A group of flagellated green algae whose constituent cells are strikingly similar and which illustrate the unicellular, colonial, and multicellular levels of organization. The small cells in *Pleodorina* and most of the cells in *Volvox* are incapable of reproduction. The scale of the drawings diminishes from left to right.

Differentiation is the acquisition of specialized structures and functions

the various parts of the shoe factory break down, the output of the factory will be adversely affected. In multicellular organisms, too, there must be proper communication and coordination between all parts. We do not know just how this is accomplished in *Volvox*. We do have ideas of how it is accomplished in many of the more elaborate multicellular forms. Part V of this book will be largely devoted to the topic of how the parts of an organism communicate and coordinate their diverse activities.

Our shoe factory analogy can be pushed one step further. If the workers in any one production department go out on strike, the output of shoes from the factory will soon cease. In an analogous way, if one group of specialized cells in a multicellular organism ceases to carry out its function, all the other cells of the organism will be affected. If the human heart fails, every other cell in the body will soon die no matter how vigorously and efficiently each had been functioning at the time.

Specialization leads to greater efficiency in exploiting the environment

You might well argue that a multicellular organism like man is really no more efficient at carrying out life's activities than a *Chlamydomonas* cell. In the sense that *Chlamydomonas* seems to be in no more (perhaps even less) danger of extinction than man, this is true. However, a comparison of its habitat with that of man may reveal what is really gained by specialization and division of labor. *Chlamydomonas* is almost entirely at the mercy of its environment. About its only means for coping with any adverse change in its environment is to swim away from it or form a resting stage until such time as conditions improve. Many changes in its environmental conditions (e.g. temperature or chemical changes in the water) can cause its death. Thus the habitat of *Chlamydomonas* is a very narrow one and it is at the mercy of it. Man, on the other hand, can live successfully in a great variety of habitats. When conditions change, he is usually able to cope success-

fully with them. Changes in temperature, diet, etc., have remarkably little effect upon the individual cells of which his body is composed. Some of the mechanisms by which man and other multicellular organisms gain such a mastery over their environment will be considered in Part III.

We cannot be certain that *Gonium, Pandorina, Eudorina,* and *Pleodorina* represent stages in the evolution of multicellular *Volvox* from unicellular *Chlamydomonas.* Whether they do or not, these organisms illustrate one way in which colonial forms may have arisen from unicellular ones and multicellular forms from colonial ones. They also illustrate the subtle shift in cell relationships that occurs as one crosses the uncertain boundary between colonial forms and multicellular, differentiated ones.

5.22 PLANT TISSUES

The cells that make up multicellular organisms become differentiated in a large, but not unlimited, number of ways. If we examine a mature vascular plant, we can identify several distinct cell types. These are grouped together in tissues. Some tissues consist of only one cell type. Some consist of several.

New cells are produced in meristems

1. Meristematic. The chief function of meristematic cells is mitosis (Fig. 5.17). The cells are small and thin-walled, with no central vacuole and no specialized features. They are located in tissues (the apical meristems) at the growing points of roots and stems. In some plants, a ring of meristematic tissue, the cambium, is also found within the mature stem. Mitosis in the meristems produces new cells for the growth of the plant. The cells produced by the meristems soon become differentiated into one or another of several types.

2. Protective. The cells of protective tissues are found on the surface of roots, stems and leaves. They are flattened cells whose top and bottom surfaces are parallel but whose sides may be arranged quite irregularly (Fig. 5.17). These cells provide protection to the cells lying beneath.

3. Parenchyma. Parenchyma cells are widely distributed throughout plants. They are large, thin-walled cells and usually have a central vacuole. Frequently they become partially separated from one another and gas fills the resulting intercellular space. They are liberally supplied with plastids. In areas not exposed to light, colorless plastids predominate and food storage is the main function. The cells of the white potato are parenchyma cells. In areas where light is present, e.g. in the leaf, chloroplasts predominate and photosynthesis is the main function.

4. Collenchyma. Collenchyma cells have thick walls that are especially developed at the corners of the cell (Fig. 5.17). These cells provide mechanical support for the plant. They are most frequently found in regions of the plant that are growing rapidly and need to be strengthened. The petiole ("stalk") of leaves is usually reinforced with collenchyma cells.

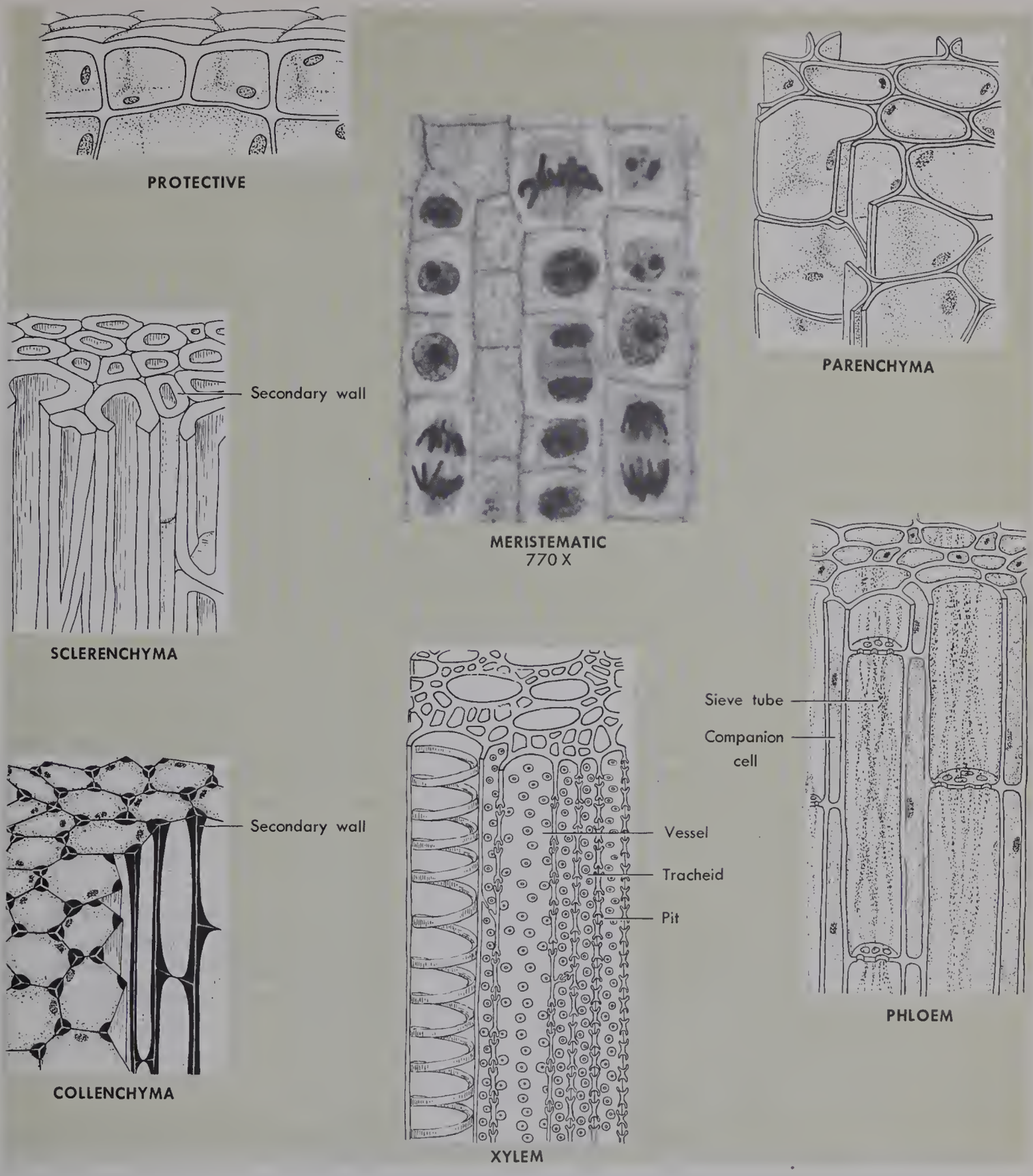


Fig. 5.17 Plant tissues. Each of these specialized types develops from the cells produced in meristems. The dark, horseshoe-shaped structures in the meristematic cells are chromosomes. In most cells they can be made visible only at the time of cell division. (Photo courtesy of General Biological Supply House, Inc.)

Collenchyma and sclerenchyma provide mechanical support

5. Sclerenchyma. Sclerenchyma cells are a more common type of supporting cell. The walls of these cells are very thick and are built up in a uniform layer around the entire margin of the cell (Fig. 5.17). Sclerenchyma cells are usually found associated with other cell types and give mechanical support to them. In many cases, the protoplasts of sclerenchyma cells die after the cell wall is fully formed. Sclerenchyma cells are found in stems and are also associated with the veins of leaves. They are the exclusive component of the hard outer covering of seeds and nuts.

Xylem transports water and gives support to the plant

6. Xylem. The xylem is a "mixed tissue" composed of several cell types. The most characteristic and important of these are the xylem vessels and xylem tracheids. (The xylem of ferns and conifers contains only tracheids.) Xylem vessels have thickened cell walls. The walls are not deposited in a uniform layer but are usually thickened in a pattern of spiral bands (Fig. 5.17). When fully developed, the end walls of xylem vessels dissolve away and the protoplasts die. This produces a long tube. Tracheids differ from vessels in that the cells lack spiral banding and are tapered at the ends. The tapered ends of vertically stacked tracheids overlap and are interconnected by means of many pits. Both tracheids and vessels are used to transport water and minerals from the roots to the leaves. In woody plants, the older xylem ceases to participate in transport and simply serves to give strength to the trunk of the growing plant. Wood is xylem. When one counts the annual rings of a tree, one is counting rings of xylem.

The phloem transports food and hormones

7. Phloem. The phloem is also a mixed tissue. The most important cells in it are the sieve tubes. They are so named because their end walls become perforated (Fig. 5.17). This allows cytoplasmic connections to be established between cells. These presumably aid the cells in carrying out their chief function: the transport of food and hormones throughout the plant. At maturity, sieve tubes do not possess a nucleus. Adjacent to them are nucleated "companion cells," however, and these may take over general control of the sieve tube cells. Frequently sclerenchyma cells also occur in phloem tissue and impart strength to it.

The various plant tissues are themselves organized in definite patterns. The groups of organized tissues make up the organs of the plant. Roots, stems, and leaves are the major organs of higher plants. Their proper functioning depends upon the proper arrangement and coordination of the tissues of which they are composed.

5.23 ANIMAL TISSUES

The cells of animals are also differentiated into a wide variety of specialized types. These diverse types are organized into tissues. In man, the following kinds of tissues can be distinguished.

1. Epithelial. Epithelial tissues are made up of closely packed cells arranged in flat sheets. These tissues line the various cavities and tubes of the body. They also form the skin which covers the body.

Protective functions

Epithelial tissues carry out a variety of functions. In every case, these functions reflect the fact that epithelia are always found at the boundary between cell masses and a cavity or space. The epithelium of the skin protects underlying tissues from damage by mechanical abrasion, ultraviolet radiation, dehydration, and bacterial invasion. In carrying out these protective functions, the surface layers of epithelium may become damaged and lost. This is especially obvious when the skin "peels" after it becomes sunburned. Fortunately, mitosis in the underlying cells replaces this lost epithelium. In fact, extra thick layers of epithelium (calluses) may form in areas where mechanical abrasion is particularly severe.

Transport functions

Epithelia also function in the transport of materials to and from the tissues and the cavities that they separate. The columnar epithelium of the intestine secretes digestive enzymes into the intestine and also absorbs the end products of food digestion from it. All the digestive glands of the body are lined with epithelium. Epithelium also lines the air tubes and lung cavities. This epithelium secretes mucus to keep from drying out and to trap inhaled dust particles. Many of its cells possess cilia on the "free" surface which propel the mucus with its load of foreign matter back up to the throat.

The sex cells must be released from the body in order to function in sexual reproduction. Thus we find that they, too, arise from an epithelium, in this case, the germinal epithelium. The countless millions of sperm cells produced by mature human males arise by repeated cell division in this tissue.

The cells of connective tissues are embedded in a matrix of extracellular material

2. Connective. Several types of connective tissue are found in the human body. Each of these consists of specialized cells imbedded in a great deal of extracellular material. This **matrix,** as it is called, is secreted by the cells. **Supporting** connective tissue is used to give strength, support, and protection to the soft parts of the body. **Cartilage** and **bone** are the two kinds of supporting connective tissue found in man. The matrix in cartilage is a protein-polysaccharide mixture, called chondrin. Our outer ear gets its shape from its cartilage skeleton.

The extracellular matrix in bone contains both protein fibers of collagen and mineral deposits. The main component of the latter is calcium phosphate, $Ca_3(PO_4)_2$, although magnesium, carbonate, and fluoride ions are also found. The living cells embedded in the bony matrix are connected with one another by cytoplasmic extensions. These cells receive their blood and nerve supply by way of Haversian canals which penetrate throughout the bone. The bone cells and their bony matrix are arranged in concentric rings around these canals (Fig. 5.18).

Binding connective tissue serves, as its name suggests, to bind body parts together. **Tendons** connect muscle to bone. The matrix is principally the protein collagen and the fibers are all oriented parallel to each other. This gives great strength to the tissue. Tendons are not elastic, however. **Ligaments** attach one bone to another. In addition to collagen fibers, ligaments contain the protein elastin. This protein permits the ligaments

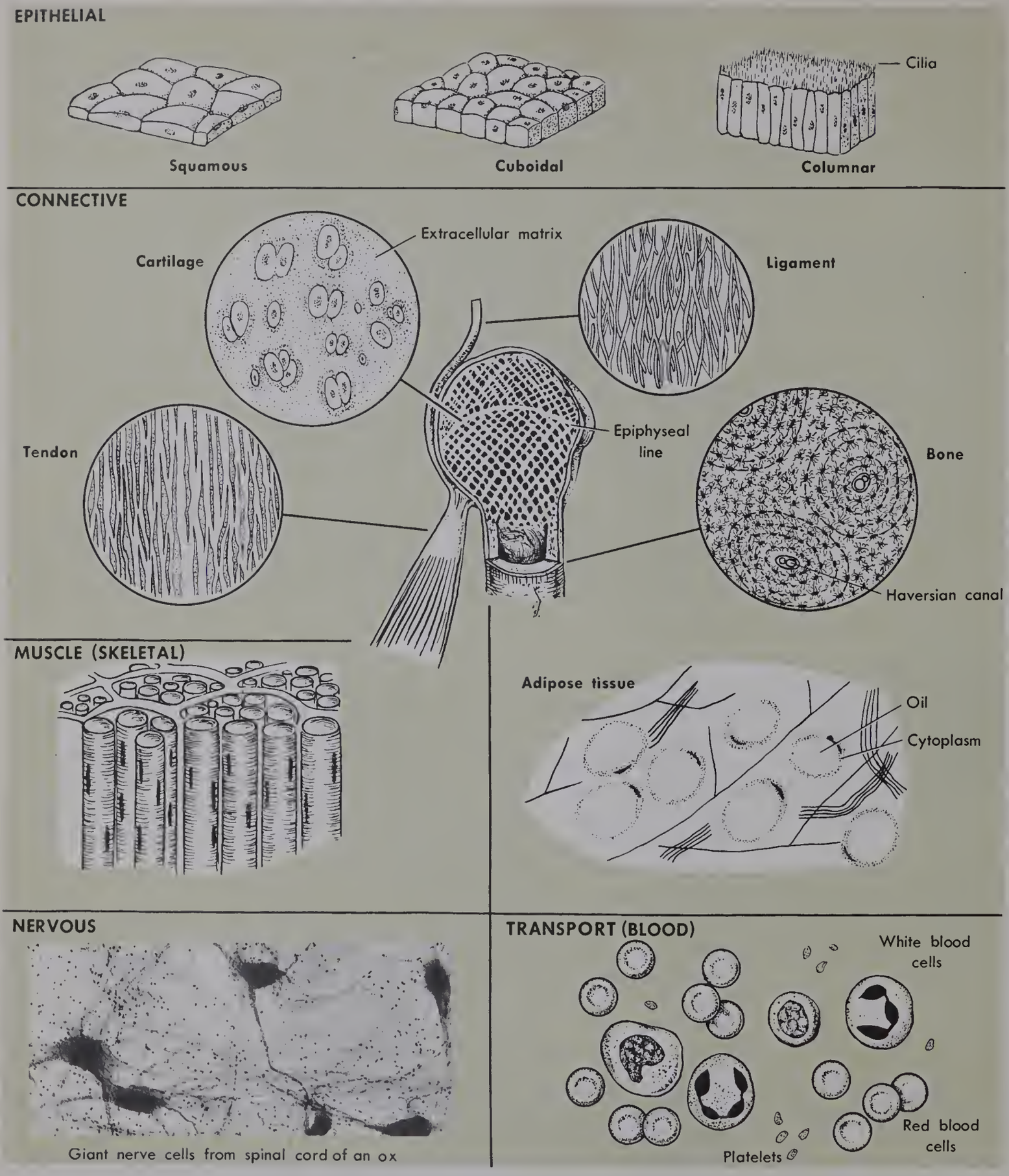


Fig. 5.18 Animal tissues.

to be stretched. If ligaments become torn or damaged, a dislocation of the joint may result.

Fibrous connective tissue is found distributed throughout the body. It serves as a packing and binding material for most of our organs. It also provides a pathway for blood vessels and nerves to reach them. Collagen and other proteins are found in the matrix. Fascia is fibrous connective tissue which binds muscles together and binds the skin to the underlying structures. Adipose tissue is fibrous connective tissue in which the cells have become almost filled with oil.

Three kinds of muscles are found in vertebrates

3. Muscle. Three kinds of muscle tissue are found in man. **Skeletal** muscle is made up of long fibers whose contraction provides the force for locomotion and other types of voluntary body movements. **Smooth** muscle lines the walls of all the hollow organs of the body such as the intestine and blood vessels. Its contraction, which is involuntary, reduces the size of the body's hollow organs. **Cardiac** muscle is the muscle out of which the heart is made. It is capable of rhythmic contraction without any outside source of stimulation.

4. Nervous. Nervous tissue is composed chiefly of **neurons.** These are cells that are specialized for the conduction of electrochemical nerve impulses. Each neuron consists of a cell body, which contains the nucleus, and one or more hairlike extensions. It is along these extensions, which in a few cases may extend several feet, that the nerve impulse travels. The tips of the extensions either meet other neurons (Fig. 5.18) or some other kind of tissue, e.g. muscle, that is stimulated by the neuron. The structure and organization of the several kinds of neurons found in the human body will be described in Chapter 27.

A neuron is a single cell

A nerve is a cable containing the extensions of many neurons

The human brain and spinal cord consist chiefly of nervous tissue. The cell bodies of virtually all our neurons are found here. Their extensions, bundled together in **nerves,** extend out from the brain and spinal cord to all parts of the body.

5. Transport. Another important tissue of our body is **blood,** a fluid tissue. Aside from its special consistency, blood is just as much a tissue as any other. Suspended in a watery matrix, the plasma, are several types of cells. Most numerous are the red blood cells which are vital to the transport of gases. White blood cells play an important role in protecting the body from disease. Platelets are tiny cell fragments that initiate the process of blood clotting when breaks or leaks occur in the blood vessels. The liquid tissue solidifies and blood loss is stopped.

The matrix of blood is plasma

We have examined the most common tissues of the human body. In every case, these are made up of differentiated cells specialized to carry out one or, at most, a few functions. In some tissues, only one type of cell predominates. Other tissues contain several cell types. Where several types are found, however, they are organized in such a way that the overall function of the tissue can be accomplished efficiently.

5.24 HIGHER LEVELS OF ORGANIZATION

The various tissues of the body are not arranged haphazardly either. Different tissues are organized and integrated into **organs.** The stomach, the liver and the heart are just three of many organs in the body. The stomach is an organ made up of epithelial, connective, and muscle tissue. Its proper functioning and coordination also depend upon nervous tissue and blood.

Societies
↑
Organisms
↑
Systems
↑
Organs
↑
Tissues
↑
Cells
↑
Organelles
↑
Macromolecules
↑
Small molecules
↑
Atoms
↑
Atomic particles

The various organs of the body are, in turn, organized into **systems.** Each system accomplishes a major function for the body. The digestive system functions in the digestion of food. The stomach, the liver, the pancreas, and the small and large intestines are some of the organs that make up this system. The system as a whole works only if its various constituent organs carry out their functions in a well-coordinated way. The respiratory, circulatory, excretory, nervous, skeletal, muscle, endocrine, and reproductory systems are other vital systems in man. We shall examine in detail the structure and functioning of these systems in later chapters. Their proper anatomical arrangement and harmonious functioning produce the **organism.**

Although we say that the cell is the basic structural and functional unit of life, the life of an organism as complex as man requires that the cells be organized into intermediate levels of complexity: the tissues, organs, and systems. Many biologists see an even higher level of organization in some living things. When many different organisms function in a coordinated way, a **society** exists. The honeybee provides a good example of this. Solitary honeybees can never survive for long. The life of any individual bee, as well as the life of the society (colony), depends upon the properly coordinated activities of workers, drones, and queen. Human societies also depend, although to a lesser degree, upon specialization and division of labor among the individuals who belong to it. Except for the differences between the sexes, we do not, however, have special body types appropriate to particular functions as do the honeybees. Furthermore, if a society does represent a higher level of biological organization, its components are surely far less rigidly organized than are the cells, tissues, organs, and systems of which individual organisms are composed.

One of the principal features of living things is the complex organization of the matter out of which they are made. In these chapters we have examined the various levels of this organization from the protons, neutrons, and electrons out of which all matter is made to the organ systems without which our most complex animals and plants could not function. Our understanding of the structure and function of any level depends upon our understanding of the structure and properties of earlier levels. Thus we understand the functioning of the organism in terms of its systems which, in turn, are understood through a knowledge of the organs, tissues, cells, and even the molecules, atoms, and subatomic particles of which they are composed.

The organization of life is not a static, unchanging thing. To be alive is to constantly build, tear down, and rebuild the structural materials out of which the organism is composed. This building up and tearing down depends upon the exchange of materials between the organism and its environment. The exchange of materials and the chemical transformations carried out on these materials within the organism constitute its **metabolism.** It is the topic we shall study in Part III.

EXERCISES AND PROBLEMS

1. In what ways do typical plant cells differ from animal cells?
2. List all those structures found in a rat liver cell that seem to be constructed from "unit membranes."
3. What are the advantages of specialization and division of labor among the cells of an organism? What are the disadvantages?
4. Distinguish between organ and organism.
5. Assuming them to be spherical in shape, compare the surface areas of a mycoplasma, staphylococcus and human cheek cell (see Fig. 5.2).
6. How would their *volumes* compare if they were all spherical?
7. What organic molecules are used in the construction of the (a) plant cell wall, (b) chromosome, (c) ribosome, (d) cell membrane, (e) lysosome, (f) oil droplet?

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Life on this earth runs with energy from the sun that is trapped by photosynthetic organisms living on land and in the sea. (Courtesy of American Airlines.)

Part III METABOLISM



6 THE METABOLISM OF CELLS

The Cellular Environment

The Composition of the ECF

Diffusion

Osmosis

Active Transport

Endocytosis

Exocytosis

Enzymes

Among the fundamental characteristics of living things discussed in Chapter 1-was metabolism. Metabolism is the exchange of matter and energy between the organism and its environment and the *transformation* of matter and energy within the organism.

An understanding of the metabolism of living things depends on an understanding of the metabolism of the individual cells of which they are made. Each cell, to survive, must secure matter and energy, and release the waste products of these transformations back to the environment.

6.1 THE CELLULAR ENVIRONMENT

Every living cell is bathed by extracellular fluid

What is the environment of cells? All living cells are bathed by liquid. This statement may seem perfectly obvious when one considers organisms that live in fresh or salt water. But what of the many organisms that live in a terrestrial environment—in the earth, on the surface of the earth, or occasionally flying above the earth? A close examination reveals that in all these cases, too, each living cell is continually bathed in liquid. The skin which man and his fellow vertebrates expose to the air consists of layers of dead cells which protect the living cells beneath from the drying effects of the air. Where living cells are exposed to the environment, as in the epithelium that lines our air passages and the transparent cornea at the front of our eyes, secretory cells bathe the exposed surfaces in a continuous supply of moisture. In a similar way, the exoskeleton of insects, the bark of trees, and the waxy cuticle of leaves all consist of dead cells or waterproof secretions of cells which permit the underlying living cells to remain protected by at least a film of moisture.

Within the soil itself, living cells may be directly exposed to the environment, but here again the environment is liquid. Each soil particle is surrounded by a film of moisture. The delicate root hairs of vascular plants, and myriads of microorganisms and tiny invertebrates that live in the soil are in contact with this moisture.

In considering the cellular environment of more complex plants and animals, one must consider also the environment of the cells that are not close to the exterior of the organism. These deep-lying inner cells are also in contact with liquid. Sap in plants, the blood in insects, and lymph in man are examples of fluids that bathe the inner cells of higher organisms. Lymph and the blood plasma from which it is derived make up about 20% of the body weight of man. Because these fluids are outside the cells, we will refer to them as the *extracellular fluid* or **ECF.** For the living cells of our body, the ECF *is* the environment. To distinguish between the external environment of our bodies (air) and the actual environment of our cells, the French physiologist Claude Bernard a century ago referred to the latter as the *internal environment.* He studied its properties carefully and found them to be remarkably stable. This is particularly true of the most complex of the multicellular organisms, the birds and mammals. Whether placed in warm locations or cold, whether recently fed or starved, no matter what kind of food taken in the diet, he found that the composition of the ECF

Claude Bernard: the "father of physiology"

remains relatively unchanged. In a real sense, the most adaptable of our higher animals actually are made of cells whose environment, the ECF, remains unchanged despite wide fluctuations in the external environment. Bernard was able to discover several mechanisms by which the mammalian body is able to maintain this constancy of its internal environment. He was so struck by these findings that he wrote: "The constancy of the internal environment is the condition of a free and independent life."

In the years since Bernard's discoveries, physiologists have studied the internal environment of other kinds of animals and found that they, too, have mechanisms for maintaining a stable ECF. The less complex animals are, however, less capable in this respect, and this may well account for the more restricted lives that they live. In the early twentieth century, the American physiologist Walter Cannon discovered still other mechanisms by which the stability of the internal environment is maintained. He coined the word **homeostasis** to describe this constancy of the ECF. Throughout Part III, many examples of homeostasis and the mechanisms that maintain it will be described.

Homeostasis is the maintenance of the constancy of the internal environment

6.2 THE COMPOSITION OF THE ECF

Water is the solvent for all the components of the ECF

1. Water. The most obvious component of the ECF of any organism—animal, plant, or microorganism—is water. This substance is uniquely suited for the functions it must carry out as the chief component of the ECF. It is unsurpassed as a solvent. In this role it brings to the cell many of the other molecules and ions that make up the ECF and without which life would be impossible. Not only does water serve as the medium in which substances are carried to and away from cells, but it also enters cells and plays a vital role in the chemical activities within.

2. Gases. The ECF also contains gases, the most important of which are oxygen and carbon dioxide. Almost all living cells require oxygen and must get rid of their waste carbon dioxide. When chlorophyll-containing cells are exposed to the light, they take carbon dioxide from and release oxygen to the ECF.

Essential inorganic ions

3. Minerals. The ECF also contains dissolved minerals, or salts, in the form of positively and negatively charged ions. Figure 6.1 lists most of the ions that are found in the ECF of animals and plants. Some, such as calcium (Ca^{++}), potassium (K^{+}), sodium (Na^{+}), chloride (Cl^{-}), phosphate ($PO_4^{\equiv}$), and bicarbonate (HCO_3^{-}), are present in relatively large quantities in animals. Calcium ions are an essential constituent of our bones as well as of the "cement" that holds individual cells together in tissues. A proper balance of potassium and sodium ions within and without the cell is necessary for the responsiveness shown by nerve and muscle cells. The chloride ion is the major negatively charged ion in the ECF. Phosphate ions, as we shall see, are intimately involved in the distribution and use of energy within the cell. Bicarbonate ions function in the transport of

carbon dioxide and help keep the ECF from becoming too acid or too alkaline. More will be said about this function later. Plants require substantial quantities of nitrates (NO_3^-) in order to synthesize amino acids and, from them, proteins.

Substantial quantities	"Trace" quantities
Na^+	Fe^{++}
K^+	Cu^{++}
Ca^{++}	Mn^{++}
Mg^{++}	Zn^{++}
$PO_4^{\equiv}$	B^{+++}
Cl^-	Mo^+
$SO_4^=$	V^{++}
HCO_3^-	Co^{++}
	I^-
	$Se^=$
	F^-

Fig. 6.1 Inorganic ions necessary for most organisms.

Trace elements

Many other ions are required only in minute quantities. Most of these "trace elements" (e.g., Cu^{++}, Zn^{++}, Mn^{++}, Co^{++}) are either incorporated in enzymes directly or are necessary for the activation of enzymes. Iodine is incorporated in the hormone thyroxin. Small quantities of the fluoride ion (F^-) are important in strengthening the mineral portions of teeth and bone and essential for normal growth in rats (Fig. 6.2).

Some years ago, sheep growers in parts of Australia were plagued by a mysterious disease which became prevalent in their flocks. Although pastured in lush fields, their animals developed symptoms of extreme starvation and anemia (insufficient hemoglobin in the blood). Death followed frequently. The anemia suggested an iron deficiency and, sure enough, it was found that treatment with crude iron compounds often prevented the appearance of symptoms. It was soon realized, though, that it was not the iron which was preventing the disease, but tiny traces of cobalt present as impurities in the iron salts. Just one ounce of cobalt was found to be enough to maintain 800 sheep in good health for a year. Curiously, injections of cobalt failed to prevent the symptoms of the disease, but tiny traces taken by mouth worked dramatically. The answer to this riddle turned out to be that the cobalt was not being used directly by the sheep at all, but by bacteria living in their digestive systems. These bacteria used the cobalt to synthesize vitamin B_{12}, which in turn was absorbed by the sheep. You will remember that vitamins are organic substances needed by an organism but which it cannot synthesize itself from the products of protein, carbohydrate, or lipid digestion.

Not only must certain ions be present in the ECF, but the relative concentration of the various ions must often be held within rather narrow limits. When physiological experimentation is performed with organs or tissues removed from animal bodies, it is necessary to keep the specimen

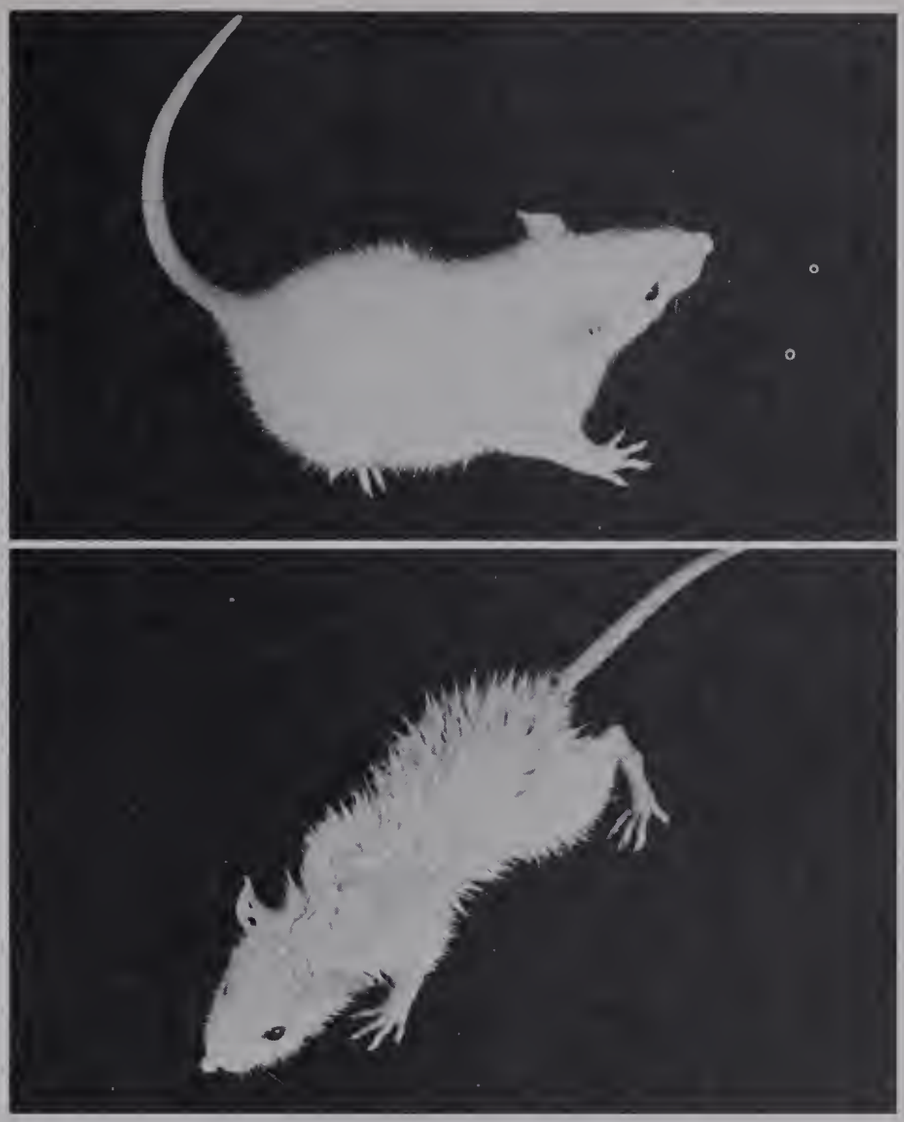


Fig. 6.2 Trace element deprivation. The rat at the bottom received the same diet as that at the top except that tin, vanadium, and fluorides were carefully excluded for 20 days. When tin and vanadium were then given to the deprived rat, normal growth still did not occur. But adding 0.5 ppm of potassium fluoride (KF) to its diet restored normal growth and health. (Courtesy of Klaus Schwarz, Veterans Administration Hospital, Long Beach, California.)

moistened with a solution which contains the same ratio of the major ions (Ca^{++}, Na^{+}, K^{+}) as is found in the ECF of the intact organism. One such solution is called Ringer's solution after the British physiologist, Sidney Ringer, who developed it.

Ringer's solution

The original discovery, as is so often the case in scientific work, was made as a result of chance. It was the practice at that time to use a simple salt solution (Na^{+} and Cl^{-} ions) for bathing tissues. On one occasion, however, a particular batch of salt solution was found to keep frog hearts alive for many times the normal length of time. When it was found that an assistant in Ringer's laboratory had made that particular solution with tap water rather than with distilled water, the search was on for the other necessary ions. Ringer's solution resulted.

Our ECF resembles dilute sea water

Another interesting fact about the ratio of ions in the ECF of animals can be seen by glancing at Fig. 6.3. Sea water, the ECF of marine invertebrates, and the ECF of vertebrates (including man) show a striking similarity in the relative amounts of the ions present. (The absolute amount of the different ions varies more widely; the concentration of salts in sea water and the ECF of marine invertebrates is roughly three times what it is in the ECF of vertebrates.) The similarity is sufficiently close that workers in the Woods Hole Marine Biological Laboratories often substitute diluted sea water for Ringer's solution in routine physiological experiments. This "Woods Hole Ringer's" serves the purpose admirably.

The similarity between the ratio of ions in sea water and in the blood plasma of animals lends strong support to the idea that life arose in the sea. Although animals now inhabit fresh water and the land, they must actively regulate the composition of their self-contained ECF so that its ionic composition continues to be compatible with the processes of life.

	Na^+	K^+	Ca^{++}	Mg^{++}	$SO_4^=$	Cl^-
Sea water	100	3.6	3.9	12.1	20.9	181
Limulus	100	5.6	4.1	11.2	13.4	187
Cod	100	9.5	3.9	1.4		150
Man	100	6.8	3.1	0.7		129

Fig. 6.3 Distribution of ions in various extracellular fluids, with all values relative to sodium (Na^+) as 100.

4. Foods and Vitamins. The ECF also contains organic compounds which serve as food or vitamins for the cells. Food substances serve as a source of energy and as a source of material for growth and repair. They include lipids, nitrogen-containing compounds such as amino acids, and perhaps most widespread of all, a carbohydrate source such as glucose. The blood plasma of man contains 0.1% of glucose or, as it is sometimes called, "blood sugar." In Chapter 25, we shall examine some of the homeostatic mechanisms by which this level of glucose is maintained within fairly narrow limits in the ECF.

5. Hormones. Hormones are an important component of the ECF of vertebrates, higher plants, and perhaps all multicellular organisms. They are chemical substances, released into the ECF by certain cells, which affect the metabolic activities of other cells in the organism. Chapters 24 and 25 will be largely devoted to a consideration of these substances.

Hormones are chemical "messengers"

6. Wastes. The waste products of cell metabolism are also components of the ECF. Among the most important to animals are the waste products of protein and nucleoprotein metabolism. These nitrogen-containing wastes, such as ammonia and urea, are somewhat poisonous. Their level in the ECF must not be allowed to exceed certain limits or death will result. In most organisms, precise homeostatic devices (e.g. the kidney) have evolved which regulate the level of these wastes in the ECF. Although

Urea is the chief nitrogenous waste of mammals

there are some cases where too low a level of a given waste in the ECF would be harmful, the major emphasis is on preventing too great an accumulation of the waste.

It is not always easy to distinguish clearly between cell wastes in the ECF and some of the chemical messengers mentioned above. A substance which is a waste product of the metabolism of one cell may well be necessary to the proper functioning of some other cell. For example, the carbon dioxide discharged into the ECF by all of our cells provides a necessary stimulus for that part of the central nervous system that controls the rate of our breathing. Here, then, is a case where too low a level in the ECF of a given waste would be as harmful as too high a level.

7. pH. An important requirement for the ECF is that it be neither too acid nor too alkaline. Degree of acidity is measured on a scale of pH units. Pure water, which is neutral, that is, neither acid nor alkaline, has a pH of 7. Acid substances are substances which produce more H^+ ions than are found in pure water. They have pH values of less than 7. Alkaline (or basic) substances are substances which combine with H^+ ions, thus leaving fewer than are found in pure water. They have pH values greater than 7 (Fig. 6.4).

Solutions with a pH of less than 7 are acidic

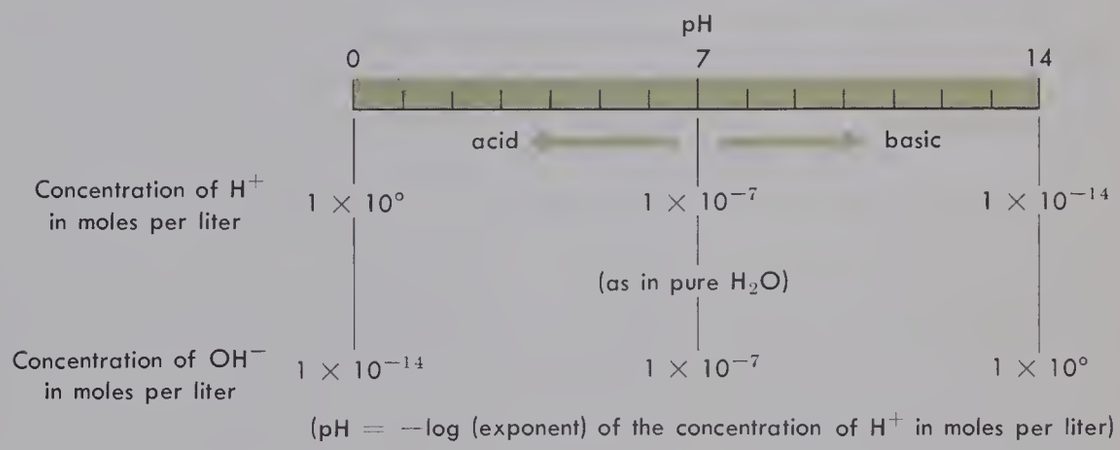


Fig. 6.4 The pH scale. A shift of one pH unit represents a tenfold shift in acidity. A solution of pH 7 is neutral.

As we saw in Chapter 3 (see Section 3.9), both OH^- ions and HCO_3^- ions combine with H^+ ions:

$$OH^- + H^+ \quad H_2O$$

$$HCO_3^- + H^+ \quad H_2CO_3 \quad H_2O + CO_2.$$

Thus, when sodium hydroxide (NaOH) or sodium bicarbonate ($NaHCO_3$) is dissolved in water, a basic solution results.

The pH scale is logarithmic: each unit on the scale represents a tenfold increase or decrease in the concentration of H^+ ions over the next lower or higher unit. For example, a solution with a pH of 5 is ten times more acid (has a concentration of H^+ ten times greater) than a solution of pH 6.

Most living cells are extremely sensitive to changes in the pH of their ECF. The pH of human plasma is usually maintained at a value between 7.34 and 7.44. If these limits should be greatly exceeded (below pH 6.8 or above pH 7.8), serious illness and perhaps death would follow. Fortu-

Buffers resist shifts in pH

nately, our plasma is well supplied with substances called **buffers,** that act to prevent any sudden shift in pH. The proteins found in blood plasma act as buffers. If the pH should start to decrease (violent exercise or holding the breath will cause this), the proteins combine with the new H^+ ions, thus keeping the pH constant. If the pH should start to rise, the proteins release H^+ ions to the ECF, thus restoring the original condition. The plasma proteins thus serve as a homeostatic mechanism for maintaining a constant pH in the ECF.

Blood plasma also contains bicarbonate ions (HCO_3^-) which serve in the same capacity. In the presence of an increased concentration of H^+ ions (and thus a decrease in pH), the following reaction occurs:

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3.$$

When the H_2CO_3 reaches the lungs, it decomposes into carbon dioxide (which passes into the air) and neutral water:

$$H_2CO_3 \rightleftharpoons H_2O + CO_2.$$

Thus the danger of too many H^+ ions in the ECF is neatly avoided.

8. Temperature. Another important characteristic of the ECF is its temperature. Microorganisms have no control over the temperature of their ECF. It is simply a function of the climatic conditions around them. This is generally true of plants, invertebrate animals, and the so-called cold-blooded vertebrates: the fishes, amphibians, and reptiles. In some of these cases, however, the organisms have a certain degree of control over the temperature of their ECF. When the temperature in a beehive becomes too high, the workers begin cooling the hive by bringing in water and fanning the air with their wings. Fanning brings in fresh air and also speeds the evaporation of the water, which is itself a cooling process.

Some animals alter the temperature of their ECF by their behavior

Goldfish have been shown to seek water in a preferred temperature range. In fact, they have been trained in the laboratory to trigger the addition of cold water when surroundings get too warm.

Many lizards also exert considerable control over the temperature of their ECF at least for the periods when they are active. Basking in the sun enables a lizard to raise its body temperature to the preferred range for its species even though the temperature of the surrounding air may be considerably cooler. When the air temperature becomes quite high, lizards can still maintain their preferred temperature by evaporating water from their tongue (panting) and retreating to shady locations.

Only mammals and birds can maintain a constant body temperature

It is among the birds and mammals, however, that we find the strictest control over the temperature of the ECF. Birds and mammals are able to maintain the temperature of their ECF within very narrow limits despite wide fluctuations in the temperature of the surrounding air. For this reason, they are often called "warmblooded," or better, **homeothermic.** A healthy human is capable of maintaining the temperature of his ECF within a degree or so of 37.5° C (98.6° F) at rest or during violent exercise, in warm surroundings or cold.

At the beginning of this chapter it was stated that metabolism involves the exchange of matter and energy with the environment and the transformation of this matter and energy within the cell. The environment of an individual cell is its ECF. We must now examine the process by which substances are exchanged between it and the cell.

6.3 DIFFUSION

Some of the materials dissolved in the ECF enter the cell by diffusion across the cell membrane from the ECF into the cytoplasm. Similarly, other substances diffuse out of the cell into the ECF.

To help us understand the process of diffusion, consider what happens when a bottle of perfume is opened in one corner of a sealed room. Even with air currents completely eliminated, the odor gradually spreads to every part of the room. Eventually the bottle of perfume becomes empty and the intensity of the odor is uniform throughout the room.

What has happened? The liquid perfume has evaporated. The molecules of which the perfume is composed have not, however, disappeared. At first they mix with the molecules of air in the vicinity of the bottle. Then, as time goes on, they spread to all parts of the room (Fig. 6.5). This process of diffusion continues until the molecules are uniformly distributed throughout the room. We can define diffusion as the net movement of molecules or ions from a region where they are in high concentration to a region of lower concentration.

The velocity of molecules and ions increases with temperature; in fact, temperature is a measure of molecular motion

What force accounts for this motion? It is believed that all the molecules in a gas or in a solution are in constant random motion. This motion takes place in straight lines and continues in a given direction until one molecule collides with another and rebounds in a different direction.

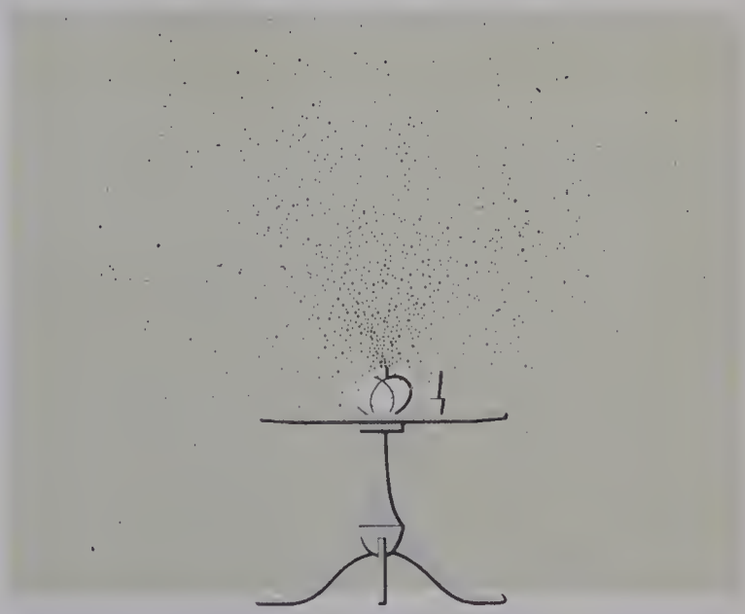


Fig. 6.5 Diffusion. Even in the absence of air currents, the perfume molecules will eventually become evenly distributed throughout the room. Their own random motion accounts for this.



Fig. 6.6 Diffusion through a barrier. As long as there is a greater concentration of molecules on the left, more molecules will pass from left to right than from right to left.

Brownian motion

One visible bit of evidence to support this theory is the phenomenon known as Brownian motion. If with a high-power microscope you examine a water suspension of tiny particles such as bacteria, you will see that the particles are in continual random motion or vibration. This motion is named after the English botanist Robert Brown, who first described it in 1827. It should not be confused with any swimming motions displayed by some bacteria. It is believed to be caused by continual collisions between the water molecules and the bacteria. It is thus an entirely physical process. Dead bacteria show Brownian motion just as well as living ones do. Why, though, do you suppose larger objects fail to show this activity?

Diffusion occurs "down" a gradient of concentration

The odds are that any object moving about entirely at random will gradually move away from its starting point. Furthermore, in the case of the perfume bottle, any molecule moving away from the bottle would be apt to travel farther than a molecule moving back towards the bottle. This is because there are more perfume molecules nearer the bottle and hence a greater chance of collisions to occur. Thus there will be a net movement of the molecules from the region of greater concentration to the region of lesser concentration. When the molecules are finally distributed evenly throughout the room, the process of diffusion ceases. This does not mean that motion of the molecules ceases. There is no longer a net movement or trend, however, and hence there is no more diffusion.

Diffusion across a membrane

In the case of the perfume bottle in the room, no barrier was placed between the region of high concentration and the region of low concentration. In the case of diffusion between cells and their ECF, a definite barrier, the cell membrane, is present. However, as long as there are pores in the membrane sufficiently large to let a given size of molecule pass through, diffusion of that substance can occur. A glance at Fig. 6.6 should make the mechanism clear. Whenever a small molecule strikes the macromolecules of which the membrane is made, it rebounds. If, however, it reaches the membrane in the vicinity of a pore, it can pass through to the other side. With a greater concentration of a given type of molecule on one side of the membrane than on the other, there will be more collisions with the membrane on that side. There will also be more successful passages through the membrane in this direction. Although molecules do pass through the membrane in both directions, diffusion is considered to occur only in the direction of greater movement.

A demonstration of diffusion

We can demonstrate the process of diffusion through a membrane in the following way. Divide a chamber into two compartments by means of a sheet of cellophane (Fig. 6.7). Fill one compartment (A) with a solution of glucose (or any small molecule or ion whose concentration can be easily measured). Fill the other compartment (B) with pure water. Then, every few minutes remove a sample from each compartment and measure the concentration of glucose in each. You will find that as time goes by, the concentration of glucose drops in compartment A and, at the same rate, rises in compartment B. Finally, a time is reached when the concentrations in the two chambers become equal (Fig. 6.7) and remain so.

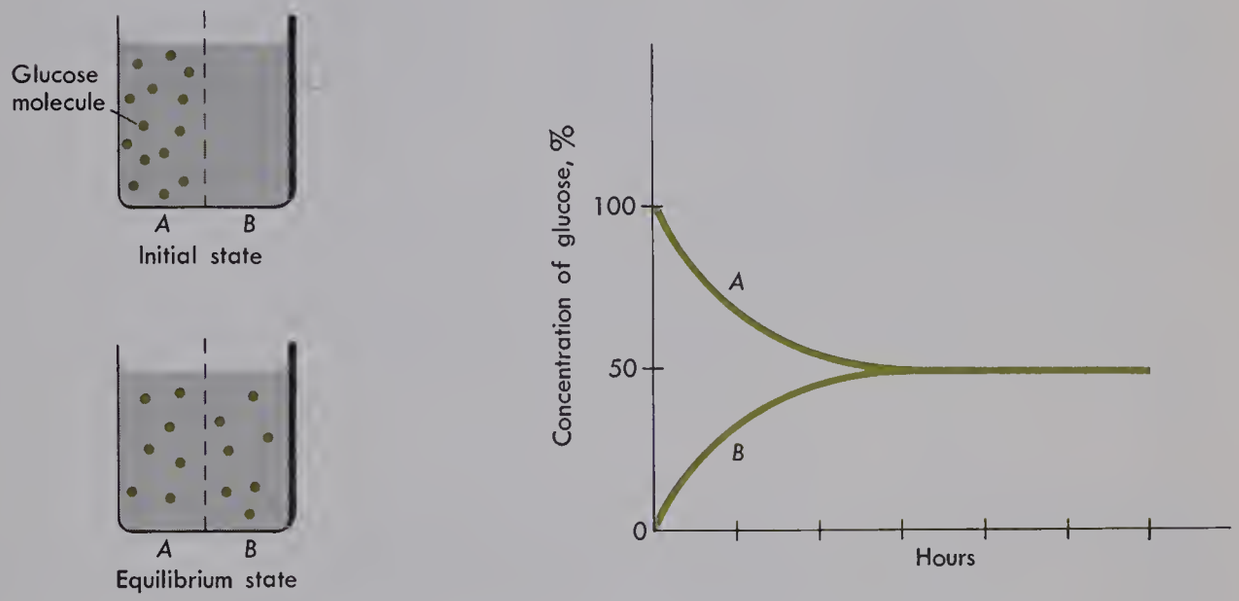


Fig. 6.7 Apparatus with which the diffusion of a small molecule across a semipermeable membrane can be measured. At the start of the experiment, all of the solute is in compartment A. With the passage of time, diffusion of the solute causes its concentration to drop in A and, at the same rate, rise in B. After 3 hours, the concentrations in the two chambers are equal and equilibrium has been reached.

6.4 OSMOSIS

The diffusion of water across a differentially-permeable membrane is called osmosis

Osmosis is simply a special case of diffusion. Chemists define osmosis as the diffusion of any solvent through a differentially permeable membrane. Cell membranes, having pores which permit the passage of some molecules but prevent the passage of others, are differentially permeable. The universal solvent in living things, as was mentioned earlier, is water. For our purposes, therefore, osmosis can be defined as the diffusion of water through a differentially permeable membrane from a region of high concentration to a region of low concentration. Note that concentration refers to the concentration of the solvent, water, and not to the concentration of molecules or ions which may be dissolved in the water. The exchange of water between the cell and its environment is such an important factor in cell function that it justifies the special name of osmosis.

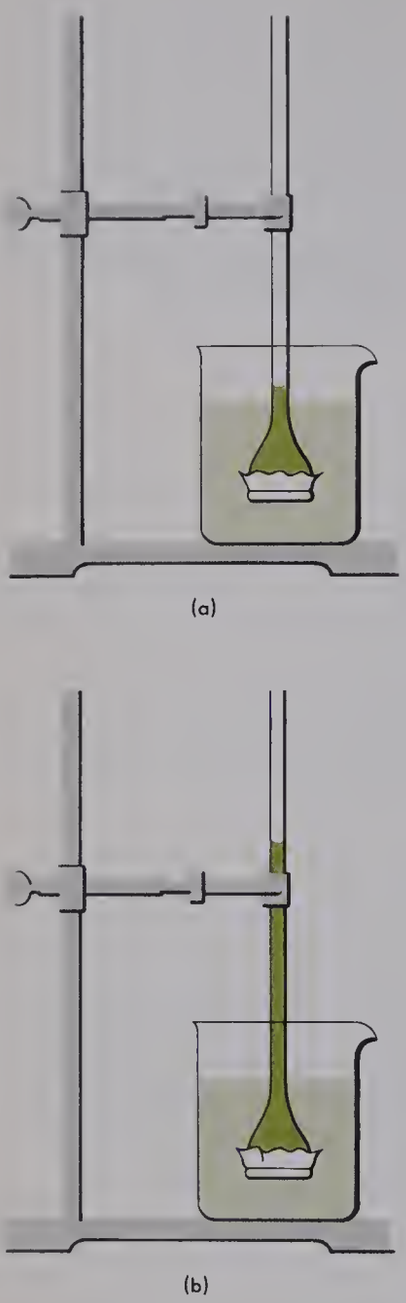


Fig. 6.8 Osmometer. (a) At start. (b) A few hours later. The cellophane is a differentially permeable membrane; water molecules pass through it more readily than do sugar molecules.

An experiment that demonstrates osmosis is shown in Fig. 6.8. The lower opening of the glass tube is covered with a sheet of cellophane. This acts as a differentially permeable membrane, permitting the rapid passage of water molecules but obstructing the passage of larger molecules. The interior of the tube is filled with molasses, a concentrated solution of sugar in water. The whole apparatus is placed in a beaker containing distilled water. In which direction will osmosis occur? The water concentration in the beaker is 100%. The water concentration inside the tube is less than this because a given volume of molasses contains fewer water molecules than the same volume of distilled water. There is, therefore, a net movement of water molecules through the cellophane membrane and into the tube.

As additional water molecules enter the tube, the volume of fluid increases. The molasses is forced up the tube. This force arises from pressure exerted by the diffusion of water molecules into the tube. The pressure is called osmotic pressure. The greater the difference in water concentration on either side of the membrane, the greater the tendency for osmosis to occur and thus the greater the osmotic pressure. In fact, when the column of molasses stops rising, we have a rough measure of the osmotic pressure of the system. The weight of the column of water finally counterbalances the osmotic pressure and osmosis ceases. Note that the water concentrations on the two sides of the membrane are still not equal by any means. However, the increase of pressure on the inner surface of the membrane, created by the weight of the column of molasses above, causes water molecules to be forced or filtered back out through the pores. When the rate at which this filtration process occurs becomes equal to the rate at which water molecules are coming in because of the difference in concentration, osmosis ceases.

Another experiment showing the effects of osmosis is depicted in Fig. 6.9. The shell membrane of a hen's egg is a differentially permeable membrane. Its pores are large enough to allow the easy passage of water molecules, but are not large enough to allow larger fat and protein molecules to get through. If after carefully removing a portion of the waterproof shell (by dissolving it away in dilute acid), one places the egg in pure water, water will diffuse into the egg. This osmosis occurs because there is a greater concentration of water outside the egg (100%) than within. The surrounding water is said to be **hypotonic** to the contents of the egg. As osmosis continues, more and more water accumulates within the egg and this crowding in of additional molecules results in a buildup of pressure. The development of pressure within a cell (and the egg *is* a single cell) as a result of the diffusion of water into it is called **turgor.** Although the remaining portion of the shell can resist the pressure, the unprotected membrane cannot. Ultimately it bursts.

What of organisms that spend their lives in fresh water? Certainly the water concentration of their cytoplasm can never approach that of the pure water surrounding them. In the case of the cells of freshwater plants, water passes into the cell by osmosis and turgor quickly develops. The strong

How freshwater plants cope with their hypotonic surroundings

cellulose walls of the cells are, however, capable of withstanding this pressure. Soon the pressure within the cell equals the osmotic pressure even though the two water concentrations are not equal. At this point, osmosis ceases (Fig. 6.9).

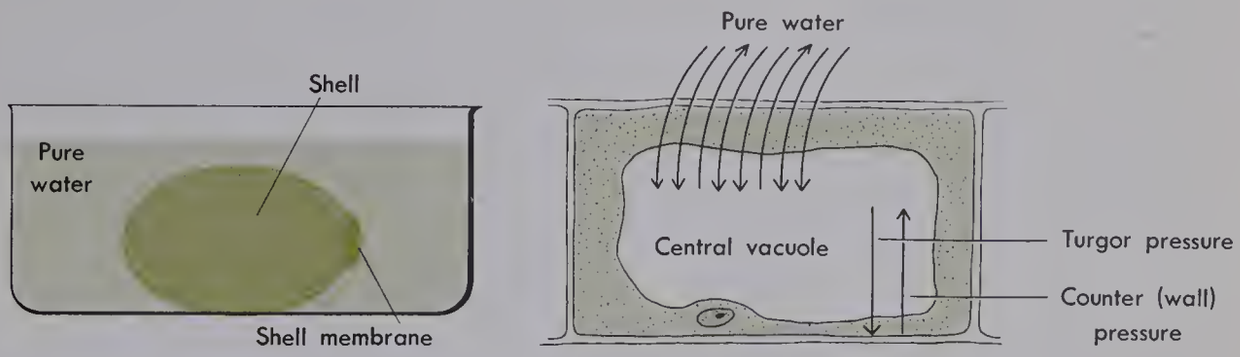


Fig. 6.9 Left: egg osmometer. The pores of the shell membrane permit water molecules to diffuse into the egg, but are too small to let the macromolecules within the egg diffuse out. Consequently, the volume of materials within the egg increases until increasing pressure ruptures the membrane. Right: turgor in a plant cell. When the pressure within the cell finally equals the osmotic pressure, the movement of water in and out of the cell will reach equilibrium.

How the amoeba copes with its hypotonic surroundings

Fresh-water animals and protozoans lack cellulose cell walls so they must cope differently with life in a hypotonic environment. Water enters their cells continually by osmosis but only slight turgor can safely be developed. The problem is solved by employing energy and some contractile structure to pump the excess water back out into the environment. The single-celled amoeba accomplishes this by means of a contractile vacuole (Fig. 6.10), in which water entering the cell by osmosis is collected. When the vacuole is filled, the amoeba contracts it (with the aid of microtubules) forcing the water out through a pore which forms momentarily in the cell membrane. Note that the water, which had entered the cell as a result of the random molecular activity of osmosis, leaves the cell by flowing out because of a force generated by the cell. The creation of this force requires the expenditure of energy by the cell. In Chapter 15, we shall examine some of the mechanisms by which freshwater animals such as fishes cope with *their* hypotonic environment.

The ECF of marine invertebrates is isotonic to sea water

Life in the oceans involves quite different osmotic conditions than life in fresh water. Sea water contains about 3.5% of various ions, especially Na^+ and Cl^-. This results in a water concentration which is approximately the same as that in the cytoplasm of marine plants and the invertebrate animals that live in the sea. Consequently, these organisms are able to exist in a state of equilibrium with respect to the water in their surroundings. They neither gain nor lose water by osmosis. We say that sea water is **isotonic** to their cytoplasm.

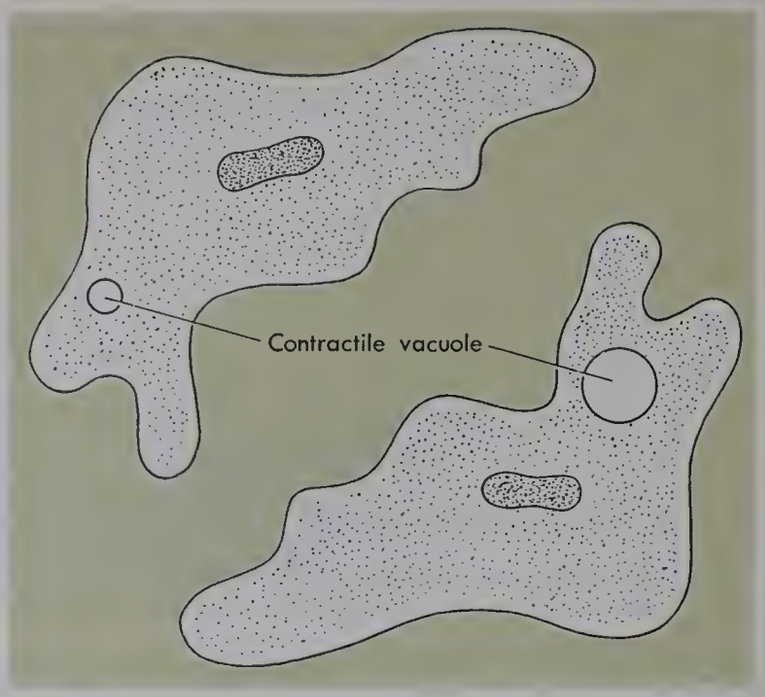


Fig. 6.10 The amoeba bails out the continual influx of water from its hypotonic surroundings by alternately filling and emptying its contractile vacuole. The contents of the vacuole may be discharged at any point on the surface of the cell.

That a given volume of sea water is about 3% salt by weight, and thus 97% water, does not mean that the water concentration of the cytoplasm of these marine plants and animals is also 97% by weight. The speed with which diffusion or osmosis takes place is a measure of the difference in the number, not weight, of the molecules or ions involved. When osmosis occurs, the region of higher concentration is simply the region which contains the greater number of water molecules in a given volume of the mixture. The cytoplasm of these marine organisms may contain as little as 80–90% water by weight. However, much of the remaining material in the cytoplasm consists of protein. These macromolecules make up a substantial fraction of the weight of the cytoplasm but contribute only a minor osmotic effect because of the relatively small number of molecules involved. Similarly, a 0.9% salt solution (99.1% water) is isotonic to human blood plasma although the latter contains only 90% water by weight. The number of water molecules in a given volume of each of the two solutions is, however, the same.

When a fresh-water (or terrestrial) plant is placed in sea water, its cells quickly lose turgor and the plant wilts. This is because a given volume of sea water contains a smaller number of water molecules than a given volume of the cytoplasm of these plants. The sea water is said to be **hypertonic** to the cytoplasm. As water continues to diffuse from the cytoplasm into the sea water, the protoplast gradually shrinks. This condition is known as **plasmolysis.** Note in Fig. 6.11 how the protoplasts have pulled away from the cell walls, which still retain their original shape.

Plasmolysis

The ECF of bony fishes has a water concentration which is substantially higher than that of sea water. Thus they live in a medium which is hypertonic. Whereas fresh-water organisms have to cope with water passing continually into their body by osmosis, the salt-water bony fish continually loses water by osmosis. Once again, however, survival depends

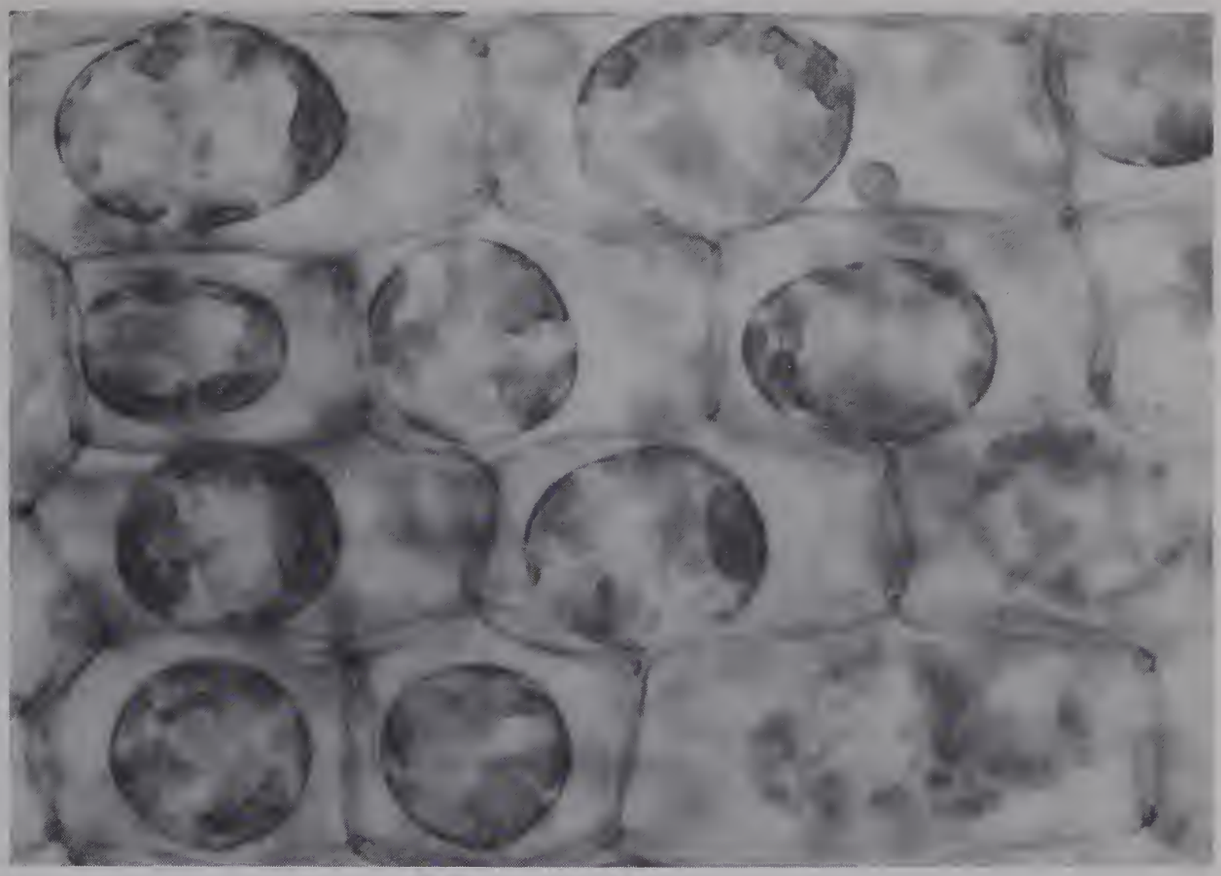


Fig. 6.11 Plasmolyzed cells in the fresh-water plant *Anacharis densa,* which has been placed in sea water. The space between the cell membranes and the cell walls has become filled with sea water.

How certain marine vertebrates cope with their hypertonic surroundings

upon the expenditure of energy to combat the force of osmosis. The fish drinks sea water and then uses metabolic energy to desalt it. The salt is transported back into the environment at the gills. Marine birds, which sometimes pass long periods of time away from fresh water, and sea turtles use a similar device. They, too, drink salt water to take care of their water needs and use metabolic energy to desalt it. The salt is extracted by two glands in the head and released (in a very concentrated solution) to the outside through the nostrils (Fig. 6.12). Marine snakes use a similar desalting mechanism.

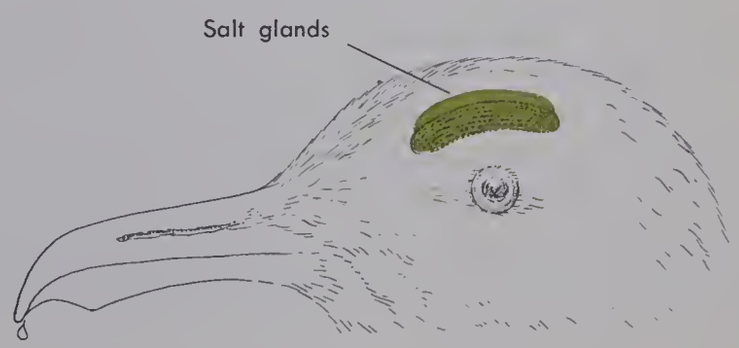


Fig. 6.12 The salt glands of the herring gull. The fluid excreted by the glands is saltier than the blood.

6.5 ACTIVE TRANSPORT

The ability of marine bony fishes and marine birds to transport ions from a region where they are in low concentration (the ECF) to a region where they are in high concentration (the ocean and the ducts of the salt glands, respectively) implies that some force other than diffusion is at work. In both cases, the transport of ions occurs against the concentration gradient and hence in a direction opposite to that which would occur by diffusion alone. This movement of ions or molecules against a concentration gradient is known as active transport.

All mechanisms of active transport require energy

It is described as active because the cell must expend energy to accomplish the transport against the passive force of diffusion. The ability of cells to accomplish active transport of ions and molecules to or from the ECF is a widespread one. Marine organisms frequently have certain ions in their cytoplasm in concentrations a thousand or more times greater than in the surrounding sea water. Iodine in brown algae and vanadium in certain marine invertebrates are found concentrated in this way. This must mean that these cells have managed to transport ions actively from the sea water to their cytoplasm.

The cells lining the intestine of the rat can transport glucose actively from a lower concentration in the intestine to a higher concentration in the blood. Most cells actively transport sodium ions out of the cell and potassium ions into the cell. The transmission of nerve impulses depends upon this phenomenon. The filling of the amoeba's contractile vacuole requires the active transport of water molecules from the cytoplasm into the vacuole itself.

The mechanism by which active transport is accomplished is not yet clearly understood. There is no doubt that the cell must use some of the energy produced during its metabolism to carry out active transport. Anything that interferes with the cell's production of energy also interferes with active transport. It is probable that specific enzymes are required which serve to carry a given molecule or ion from one side of the cell membrane to the other. In any case, the plasma membrane cannot be considered as simply a passive barrier to the diffusion of molecules. It may exert a very decided influence on what substances pass through it.

6.6 ENDOCYTOSIS

Still another mechanism by which the cell transports materials from the ECF into the interior is to engulf them by folding inward a portion of the cell membrane. The pouch that results then breaks loose from the outer portion of the membrane and forms a vacuole within the cytoplasm. In some cells, solid particles like bacteria may be engulfed in this manner. The amoeba derives its nourishment by ingesting smaller microorganisms in this fashion (Fig. 6.13). "White" cells in the blood of many animals serve the extremely valuable function of engulfing particles, such as bacteria, that may get into the animal's body. This process, which has been observed

Phagocytosis

since late in the last century, has been called phagocytosis ("cell eating"). However, in recent years it has been shown that many types of cells are able to engulf droplets of ECF by forming vacuoles, although these are too small to contain solid particles. Whatever the size and contents of the vacuole, though, the mechanism at work (which requires functioning microfilaments) seems to be the same. Therefore, the all-inclusive term *endocytosis* is now coming into common use for this process.

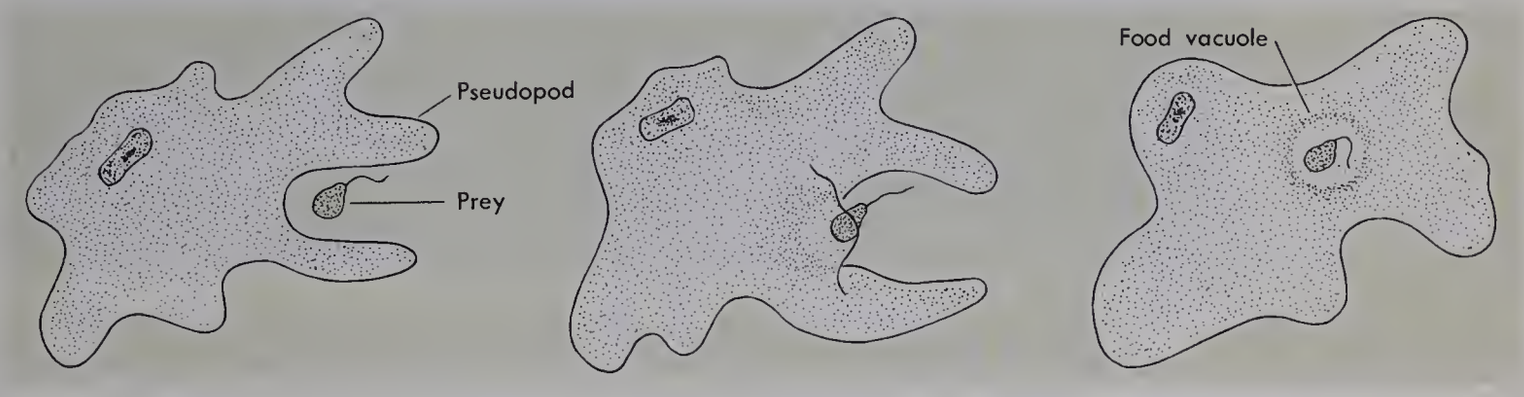


Fig. 6.13 Endocytosis in the amoeba. Even when totally engulfed, the victim is separated from the cytoplasm of the amoeba by a membrane around the food vacuole. Endocytosis of solid particles is also called phagocytosis.

Endocytosis permits molecules present in the ECF to which the cell membrane is normally impermeable to gain entry into the cell. Figure 6.14 is an electron micrograph of a section of the wall of a capillary (the smallest type of blood vessel in our bodies). At the top is the interior or bore of the capillary. In the middle is the tissue space separating the capillary wall from a nearby muscle cell (bottom). The small inpocketings of the cell membrane are clearly seen. Most of these are engulfing the ECF of the tissue space, but some can also be seen on the other side of the wall, apparently engulfing fluid from within the capillary.

Endocytosis is one kind of active transport

Endocytosis requires the expenditure of energy by the cell and enables materials to enter the cell even though they are present in the ECF in a concentration less than that within the cell. In this sense, then, endocytosis is a special case of active transport, although it is probably not the only mechanism by which active transport occurs.

The mere act of folding in a bit of the cell membrane with its content of materials from the ECF does not actually get these materials into the cytoplasm. They are still retained within vacuoles which, although surrounded by cytoplasm, are separated from it by a membrane. In the amoeba, the vacuole formed as a result of endocytosis remains intact until it is finally discharged at the margin of the cell. During the period between its formation and discharge, however, some substances are certainly exchanged between it and the surrounding cytoplasm. Digestive enzymes

Lysosomes may fuse with endocytotic vacuoles



Fig. 6.14 Endocytosis in the thin cell that forms the wall of a capillary. Note that the inpocketings have formed on both surfaces of the cell. (67,500 X, from Fawcett, *The Cell: Its Organelles and Inclusions,* W. B. Saunders Co., 1966.)

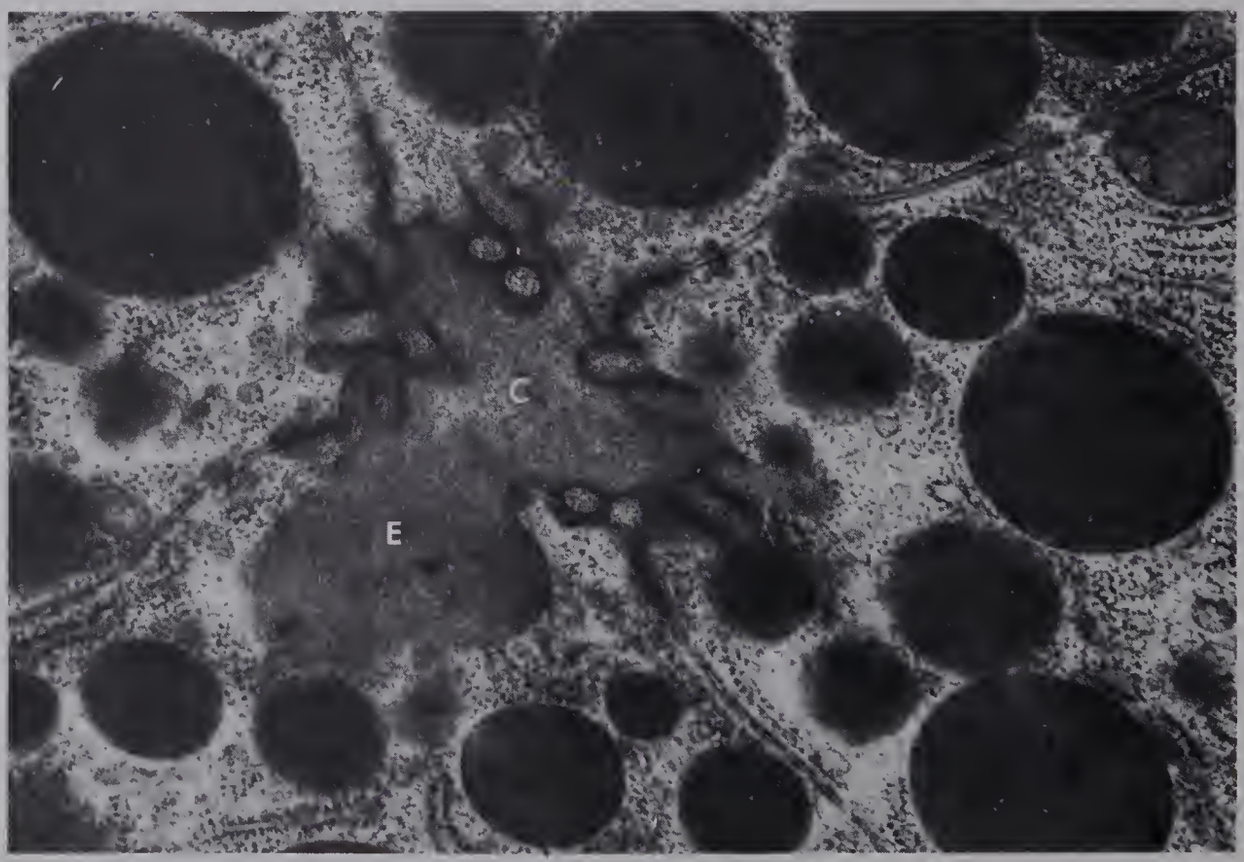


Fig. 6.15 Exocytosis. The large spherical bodies contain digestive enzymes. One is seen in the process of discharging its contents (E) into the intercellular canal (C) where the corners of these four bat pancreas cells meet. (30,000 X, from Fawcett, *The Cell: Its Organelles and Inclusions,* W. B. Saunders Co., 1966.)

enter the vacuole. This probably occurs when lysosomes (see Section 5.12) fuse with it. The small molecules produced by digestion pass through the vacuolar membrane into the cytoplasm.

6.7 EXOCYTOSIS

Cells discharge materials by exocytosis

Exocytosis is the reverse of endocytosis. The discharge of the old food vacuole of an amoeba, mentioned above, is an example. In cells that secrete large amounts of protein, the protein first accumulates in a membrane-bounded sac within the Golgi apparatus. This moves to the surface of the cell where its membrane fuses with the cell membrane, and it discharges its contents to the outside (Fig. 6.15). The cells lining our intestine synthesize tiny droplets of fat and then discharge these from the cell by exocytosis. It may even be that some of the tiny vacuoles shown in Fig. 6.14 are not taking up material by endocytosis, but are instead discharging material by exocytosis. In other words, the endocytotic vacuoles formed at one surface of the cell may, after being detached, move through the cell to the other surface and there discharge their contents. In this way, materials can be moved efficiently through the capillary wall.

CELL CHEMISTRY

The exchange of materials between a cell and the ECF is one aspect of metabolism. The chemical transformation of these materials within the cell is another. Literally hundreds of the chemical changes that occur within cells have been discovered and the list grows longer every year. In the remaining chapters of Part III several of the most common and most important of these will be described. Despite their variety, we will find that there is one feature they all share: each chemical change can occur only in the presence of a specific enzyme.

6.8 ENZYMES

In Chapter 3 we saw that even energy-yielding reactions, such as the burning of a strip of magnesium, require the input of some energy before they can proceed spontaneously. This energy is known as the energy of activation. Glucose exposed to oxygen in the air can become oxidized to carbon dioxide and water, but at room temperature the process goes on so slowly as to be imperceptible. When glucose is heated in a flame, however, a greater number of molecules receive their necessary energy of activation and the process proceeds more rapidly. Once a large number of molecules are being oxidized, the energy liberated supplies nearby molecules with their energy of activation. Soon the process becomes self-sustaining and the sugar burns rapidly.

Enzymes lower the energy of activation

The temperature necessary for this to occur is much higher than that which can be tolerated within living cells. Nevertheless, the oxidation of glucose can go on within living cells at a rapid rate and still at a low temperature. How is this accomplished? In Chapter 3 we saw that substances called catalysts are able to lower the activation energy of molecules and thus permit them to undergo rapid chemical change at lower temperatures. Respiration in living cells likewise requires the activity of catalysts to accomplish this same function. These catalysts are called **enzymes.**

Enzymes, like other types of catalysts, do not participate permanently in the chemical change that they promote. When the chemical change is completed, the enzyme is released unchanged. Because enzymes are not used up by the processes they catalyze, they may be re-used again and again. A single molecule of the enzyme catalase (see Section 3.11) is capable of catalyzing the breakdown of approximately 10 million H_2O_2 molecules every minute at the temperature of ice water (0°C)!

Enzymes are reusable

Because enzymes can be re-used, only small amounts of each enzyme are needed by a single cell. Since a single cell may be capable of carrying out over a thousand different chemical reactions, and each one of these probably requires its own special enzyme, it is fortunate that large quantities of enzymes are not necessary.



Fig. 6.16 The "lock-and-key" theory of enzyme action.

The fact that each chemical reaction in the cell requires its own special enzyme and, conversely, that a single enzyme can catalyze only one (or sometimes a few quite similar) chemical reactions requires some explanation. One theory which has been created to explain this specific relationship between an enzyme and the substance upon which it acts (its **substrate**) is called the **lock-and-key theory.** According to this theory, an enzyme actually combines with the molecule of its substrate for a brief period of time. Presumably the combination requires that each molecule have a shape complementary to that of the other. This would explain the specificity of enzymes. Different substrate molecules each possess a different shape and thus require a different enzyme. The combination between enzyme and substrate makes the substrate molecule more reactive; that is, it lowers the energy necessary for the substrate molecule to undergo chemical change. Once the chemical transformation in the substrate molecule is completed, the products leave the enzyme, which then is free to repeat the process with a new substrate molecule. Figure 6.16 shows how this process is thought to work.

Enzymes unite briefly with their substrate

There is quite a bit of experimental evidence to support the idea that the specific action of enzymes depends upon a brief union between the enzyme molecule and the substrate molecule. One type of evidence is the phenomenon of competitive inhibition of enzymes. Certain poisons exert their influence by blocking normal enzyme activity. It is thought that the shape of the poison molecule is so similar to that of the normal substrate molecule that the enzyme combines with it instead. However, the enzyme is not able to promote the chemical change of the new molecule. The poison molecule remains attached to the enzyme molecule, which is therefore no longer able to carry out its normal function. Among our vital enzymes is one that catalyzes the oxidation of succinic acid, a reaction which is an essential part of cellular respiration. The compound malonic acid blocks the activity of this enzyme. The molecular structure of malonic acid is sufficiently similar to that of succinic acid (Fig. 6.17) that it presumably combines with the enzyme but cannot then be transformed and released. The enzyme molecule is thus kept from combining with its normal substrate.

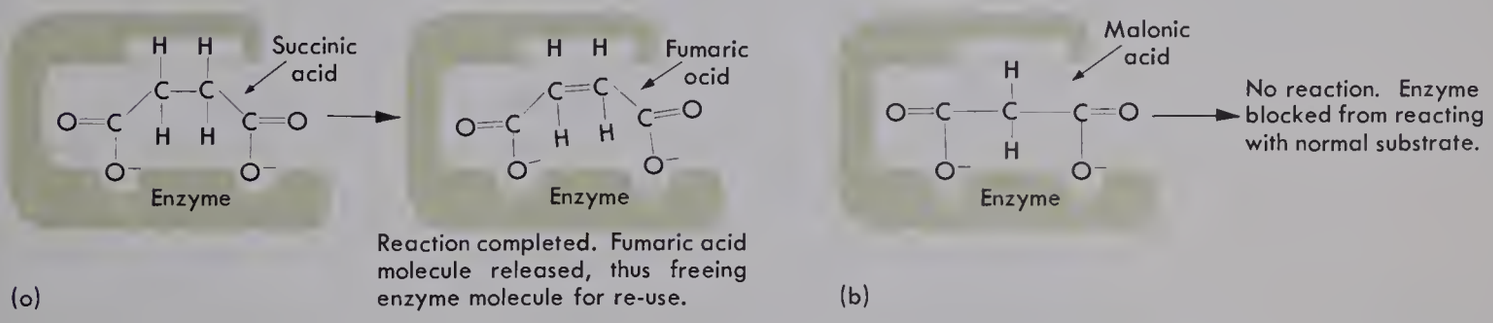


Fig. 6.17 (a) Schematic representation of the action of the enzyme, succinic dehydrogenase, on succinic acid. (b) The competitive inhibition of the enzyme by malonic acid.

One difficulty that has been encountered in trying to support this picture of enzyme action has been the problem of determining what the actual shape of any enzyme molecule is. All enzymes are proteins and hence exceedingly complex molecules. Only recently has the chemical and physical analysis of certain proteins begun to reveal their shapes. One of these is an enzyme, lysozyme, that is found in egg white and such body secretions as tears. It has a strong antibacterial action because its substrate is a polysaccharide that makes up the cell walls of bacteria.

The lysozyme molecule appears to be roughly globular in shape but with a long, narrow cleft on one surface. The long cellulose-like molecule (Fig. 4.5) of its substrate fits into this cleft. When in the cleft, one of the sugar units in the molecule is twisted out of its normal position. This seems to impart the necessary energy of activation, and the bridge between that sugar residue and the next is broken. In this way, the structure of the bacterial cell wall is broken down. (You can read an account of these discoveries in the article by Phillips listed at the end of the chapter.)

The naming of enzymes

So many enzymes have been discovered in living cells that some uniform system of naming them has become essential. Some enzymes are named by using a prefix taken from the name of the substrate and the suffix *-ase.* Thus fat-digesting enzymes are called lipases, and protein-digesting enzymes are proteinases. Some of the more recently discovered enzymes are named by giving the full name of the substrate, followed by the action carried out on the substrate and then the suffix *-ase.* Thus the enzyme which oxidizes triose phosphate (by removing two hydrogen atoms) is called triose phosphate dehydrogenase. Some of the enzymes that were discovered early in the history of enzyme chemistry still retain their early names and may not adhere to the principles just described. The protein-digesting enzymes pepsin and trypsin are two examples in this category.

Each enzyme has an optimum temperature and pH for its activity

All of the enzymes that have been discovered so far are proteins and are quickly destroyed by high temperatures. Just as boiling water will denature or "cook" the proteins in an egg, so it will quickly inactivate enzymes. Enzymes are also sensitive to change in pH. Each enzyme operates most effectively at a certain pH, its activity diminishing at values above or below that point. The protein-digesting enzyme pepsin works most effectively at a pH of 1-2 while the related enzyme trypsin is quite inactive at that pH but functions effectively at a pH of 8.

Coenzymes

Many enzymes require for their action some additional substance. This accessory substance may be the ion of some metal such as Zn^{++}, Cu^{++}, or Co^{++}, or it may be a small organic molecule called a coenzyme. The "B" vitamins, for example thiamine (B1), riboflavin (B2), and nicotinamide, function as coenzymes. Coenzymes may be tightly (covalently) bound to the protein part of the enzyme as a *prosthetic group.* Others bind more loosely and, in fact, may only bind transiently to the enzyme as it carries out its catalytic function.

Considering the large number of enzymes in the living cell, one might well ask how their activity is regulated. A number of mechanisms play a part in making enzyme action within the cell efficient and well coordinated.

For those enzymes, such as proteinases, which can attack the very substance of the cell itself, we find that their action is inhibited while they are present within the cell. The proteinase pepsin, for example, is manufactured by the cell in an inactive form, pepsinogen. Only when exposed to conditions of low pH outside of the cell is the inhibiting portion of the enzyme molecule removed and the active pepsin produced. Other potentially destructive enzymes are sequestered in the lysosomes (see Section 5.12) and thus isolated from the rest of the cell.

Many of the cell's enzymes cannot move freely within the cell but are, instead, arranged in definite patterns. The enzymes within mitochondria and chloroplasts appear to be organized spatially in such a way that they interact with the greatest efficiency. It is quite likely that spatially organized enzyme molecules are also present on the cell membrane and the membranes of the endoplasmic reticulum.

Feedback inhibition

The activity of enzymes within the cell is also closely regulated by the need for them. If the product of a series of enzymatic reactions (e.g. an amino acid) begins to accumulate within the cell, it specifically inhibits the action of the first enzyme involved in its synthesis (Fig. 6.18). Thus further production of that amino acid is temporarily halted. On the other hand, the accumulation of a substance within the cell may specifically activate an enzyme that sets in motion a sequence of reactions for which that substance is the initial substrate (Fig. 6.18). This action (which is called "precursor activation") reduces the concentration of the initial substrate to normal levels. Note that in both these situations, the activity of an enzyme is being regulated by a substance which is *not* its substrate. We might expect that the regulating molecule would have to bind to the enzyme in order to affect its function, and this is indeed the case. Interestingly, the site where the regulatory molecule binds to the enzyme is not the same as the site where the substrate binds. However, when the regulatory molecule binds to its site, it appears to alter the shape of the enzyme in such a way that its activity is altered. This interaction between regulatory and catalytic sites—brought about by a change in the shape of the enzyme—is called an **allosteric** effect. In the case of feedback inhibition, the regulatory molecule exerts its allosteric effect so that the affinity of the enzyme for its substrate is lowered. In the case of precursor activation, the situation is the opposite. The allosteric regulator molecule increases the affinity of the final enzyme in the series for its substrate (Fig. 6.19).

Precursor activation

Allosteric regulation

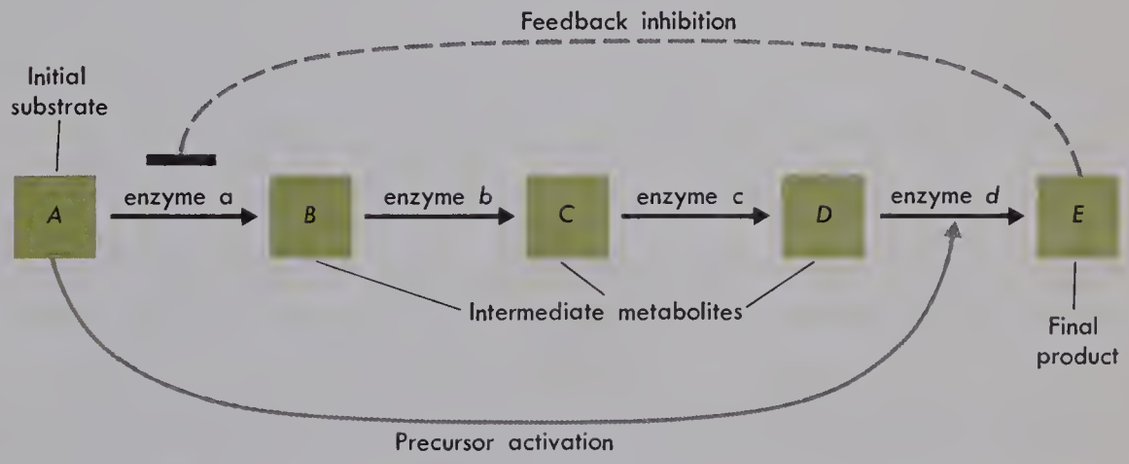


Fig. 6.18 Two distinct homeostatic mechanisms that regulate concentrations of metabolites within the cell. An accumulation of substrate A activates enzyme d, thus drawing A into the metabolic pathway. An accumulation of product E suppresses the action of enzyme a, thus inhibiting the further synthesis of E.

Regulation of enzyme synthesis

The mechanisms mentioned above ensure that the activity of enzymes already present in the cell will be properly regulated. What of enzymes that may not be needed at all or that may be needed but are not present? Here, too, delicate controls are at work. These regulate the rate at which

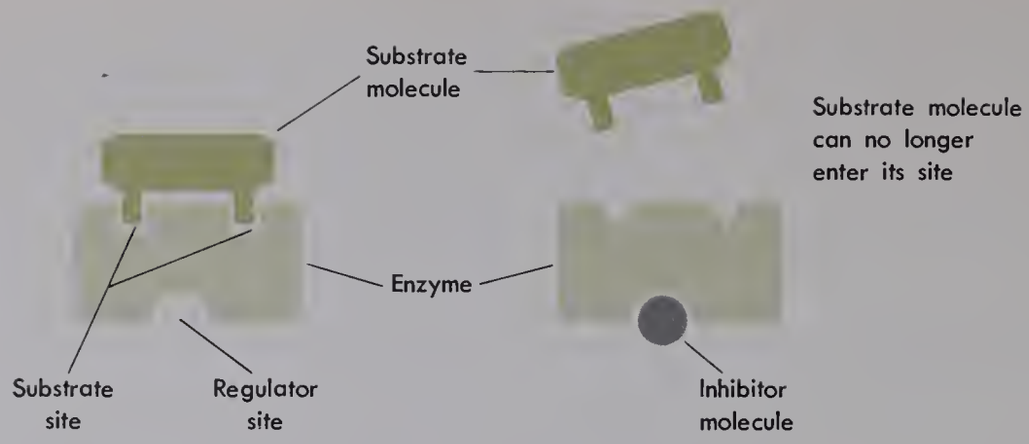


Fig. 6.19 Probable mechanism of feedback inhibition. In the case of precursor activation, the binding of the regulatory molecule would *permit* the substrate to bind to its site. In both cases, the regulator molecule has altered the affinity of the enzyme for its substrate, perhaps through causing a change in the shape of the enzyme. Such an effect is called allosteric.

new enzymes are synthesized. If, for example, excess quantities of an amino acid are supplied to a cell from its ECF, the synthesis of all the enzymes by which the cell ordinarily would produce that amino acid for itself will be halted. Thus the cell enhances its efficiency by not producing enzymes that it does not need. Conversely, if a new substrate is made available to the cell, it will stimulate the synthesis of the enzymes needed to cope with it. Yeast cells do not ordinarily ferment the disaccharide lactose and ordinarily no lact*ase* can be detected within their cells. If grown in solutions containing lactose, however, they eventually begin producing lactase and begin to metabolize the sugar.

In these cases where the *synthesis* of enzymes is being regulated, it is clear that this regulation works through the hereditary controls coded in the DNA of the nucleus. The mechanisms by which portions of the hereditary code are thought to be turned on and off in response to the needs of the cell will be examined in later chapters.

Whether acting on the enzymes already present within the cell or on the rate of synthesis of new enzymes, it is clear that these control mechanisms work together to stabilize the levels of substrates and products in the cell. In a sense, then, these mechanisms are homeostatic devices, working within the cell, that regulate enzyme activity with the utmost efficiency and in harmony with the changing needs of the cell.

Metabolism in plants and algae includes the manufacture, by photosynthesis, of organic molecules from inorganic ones taken in from the environment. The nutrition of these organisms is said to be **autotrophic.** Animals and all the other organisms that lack chlorophyll must secure their organic molecules from the environment. This form of nutrition is referred to as **heterotrophic.** The way in which heterotrophic organisms secure their food molecules is the topic of the next chapter.

EXERCISES AND PROBLEMS

1. Describe three different ways in which materials in the human intestine might enter the cells lining it.
2. After the molasses reaches its maximum height (see Fig. 6.8), what will happen? Why?
3. Which will have the higher osmotic pressure, a 1-molar solution of glucose or a 1-molar solution of salt? Why?
4. A single molecule of catalase can decompose ten million molecules of H_2O_2 each minute at 0° C. What would you expect the approximate figure to be at 10° C?
5. What would happen if (a) red blood cells, (b) plant cells, (c) an amoeba were placed in distilled water? Explain.
6. What would happen if (a) red blood cells or (b) plant cells were placed in sea water? Explain.
7. What would happen if an amoeba were placed in an isotonic solution?
8. If a mole (28 g) of nitrogen molecules (N_2) and a mole (28 g) of ethylene molecules (CH_2CH_2) were released on opposite sides of a partition that divided a room into equal volumes and then the partition were removed, do you think diffusion of the molecules would occur? Explain.

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7 HETEROTROPHIC NUTRITION

Requirements

Intracellular Digestion

Extracellular Digestion

A Filter Feeder: the Clam

Active Food Seekers: the Grasshopper and the Honeybee

The Digestive System of Man

All organisms require a fairly steady supply of materials and energy from the environment in order to stay alive. For many, the chief supply of materials and the only supply of energy come from fairly complex, energy-rich, organic molecules secured directly or indirectly from the environment. (What is the ultimate source of these molecules?) Nutrition that involves dependence upon preformed organic molecules is called **heterotrophic** nutrition, and the organisms using this kind of nutrition are called heterotrophs. The nonchlorophyll-containing microorganisms, the few nongreen plants, and all animals are heterotrophic.

Definition of heterotrophic nutrition

7.1 REQUIREMENTS

The organic molecules that serve as a source of material and energy are the sugars, amino acids, fatty acids and glycerol, and (for purposes of synthesis only) the vitamins. Not all heterotrophs depend upon all these organic molecules. Some microorganisms, for example the bacterium *E. coli,* thrive with just sugar as their source of energy. They must, however, take in some inorganic materials such as nitrates in order to synthesize all their other organic constituents. Man is quite demanding in his requirements for preformed organic molecules. He requires carbohydrates, eight of the twenty amino acids found in his body proteins (these are the "essential" amino acids—from them he can synthesize the other 12), and probably eight or more vitamins. Although he can manufacture fats from sugars, he seems to need certain special fats in his diet also.

7.2 INTRACELLULAR DIGESTION

Solid food materials are usually broken down into a solution of relatively small, soluble, organic molecules before they can be used by heterotrophic organisms. This breakdown process is called **digestion.** In some heterotrophic organisms, digestion is intracellular, that is, it occurs after the solid material has actually been engulfed by a cell.

The amoeba engulfs solid particles such as smaller protozoans by endocytosis (see Section 6.6). The prey, with a small amount of the ECF, is incorporated into a **food vacuole** within the cytoplasm of the amoeba. Next, the digestible portions of the food material are digested by enzymes secreted into the vacuole from lysosomes that fuse with it (Fig. 7.1). The soluble food molecules then pass through the vacuolar membrane into the rest of the cell. Indigestible parts are eventually discharged to the outside. Although digestion in the amoeba can properly be described as intracellular, we should keep in mind the definite membrane that persists between the material in its food vacuole and the remainder of the cytoplasm.

The enzymes within lysosomes accomplish intracellular digestion

It is obvious that endocytosis can occur only if the food materials available to the organism are smaller than its endocytic cells. It is not surprising, therefore, that feeding by endocytosis is restricted to those organisms that are adapted to secure food materials much smaller than themselves.

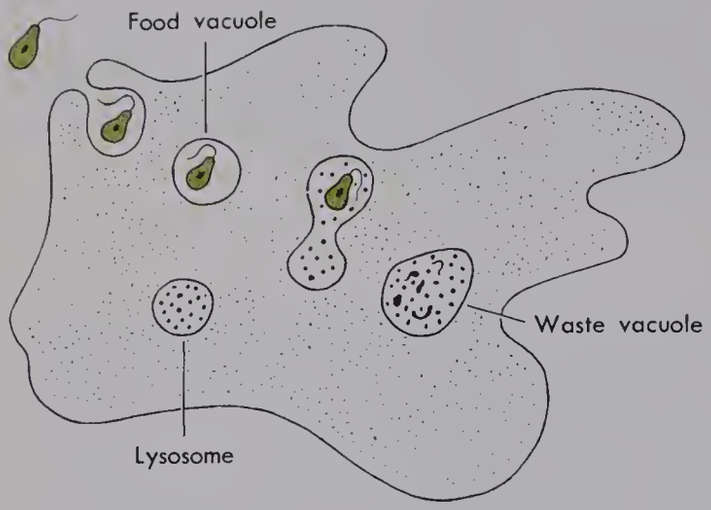


Fig. 7.1 Intracellular digestion in the amoeba. Digestive enzymes stored within lysosomes hydrolyze the food molecules of the prey into smaller molecules that can be absorbed through the vacuolar membrane into the cytoplasm.

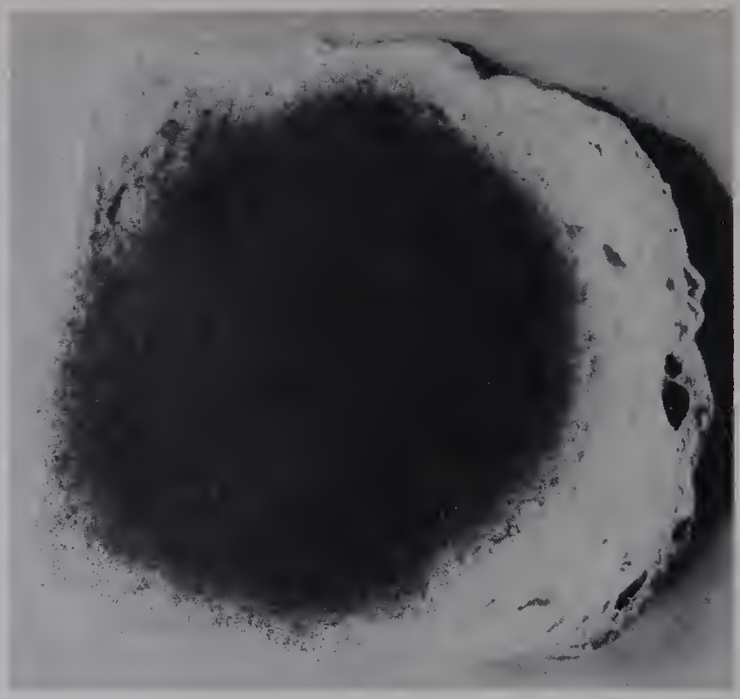


Fig. 7.2 A common mold, *Rhizopus nigricans,* growing on a piece of bread. This saprophyte secures all its nourishment by secreting digestive enzymes on the bread and absorbing the products of digestion.

7.3 EXTRACELLULAR DIGESTION

A second solution to the problem of digesting food is to secrete the digestive enzymes from the cell and digest the food outside the cell, that is, extracellularly. Once the food is digested, the small, soluble molecules produced (e.g. sugars, amino acids) can pass by diffusion or active transport across the cell membrane and into the cell.

Saprophytes secrete digestive enzymes into their surroundings

Perhaps the simplest approach to extracellular digestion is that employed by **saprophytes.** Saprophytes secure their food from nonliving but organic matter, such as dead bodies of plants and animals, food products, excrement, etc. The nutrition of the common bread mold *Rhizopus nigricans* is typical of the group. It thrives on a piece of moist bread kept in a dark location (Fig. 7.2). The bread, a man-made product of a once-living wheat plant, supplies all the dietary needs of the mold. The starch molecules in the bread are too large, however, to pass directly through the cell membrane. To convert these large, insoluble starch molecules into smaller, soluble molecules that can enter the cytoplasm requires a starch-digesting enzyme, or **amylase.** *Rhizopus* secretes this enzyme onto the bread and thus digestion occurs extracellularly. The sugar molecules produced are then absorbed into the cytoplasm. This pattern of extracellular digestion of foods in the substrate is typical of all fungi and most bacteria.

Most animals, too, digest their food extracellularly. Rarely, though, do they live in a location where they are literally surrounded by a substrate of organic matter. Instead, they secure by one means or another food

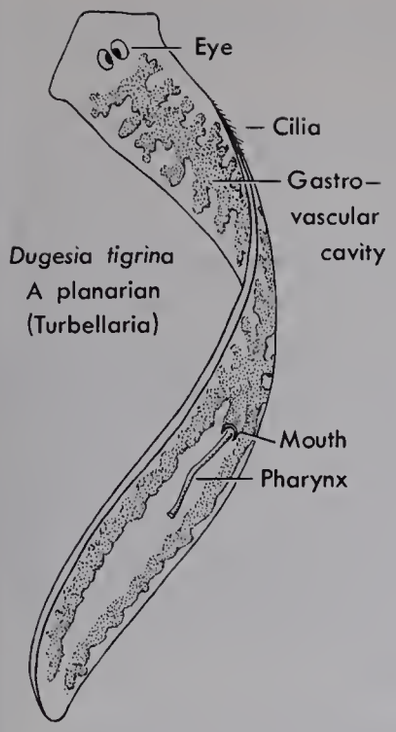


Fig. 7.3 Digestion in the planarian occurs within the gastrovascular cavity. Although branches of the gastrovascular cavity reach to most parts of the worm's body, there is no specialization of digestive functions within the cavity. Egestion, like ingestion, occurs through the mouth.

materials which appear from time to time in their surroundings. They place this food in a pouch or tube within their body, a process called **ingestion.** Then they secrete their enzymes there and digestion takes place. Thus the enzymes are localized where the food is, rather than secreted freely into the surroundings.

Digestion in the tiny freshwater planarian takes place in a highly branched pouch, the gastrovascular cavity (Fig. 7.3). Any undigested material left over leaves by the way it entered: through the mouth.

Although ingestion is described as the taking of food-containing solids into the organism, these solids are taken in only in a superficial sense. The cavity or tube that receives the solid food materials is really only a portion of the outside of the organism turned inward. A marble swallowed by a baby never enters any cell nor participates in any metabolic activity. A day or two later it emerges at the other end quite unchanged.

7.4 A FILTER FEEDER: THE CLAM

Some freshwater and many marine animals feed by taking in a stream of water and filtering from it any small organisms that may be present. These are then ingested into the alimentary canal for eventual digestion and absorption. The barnacle and the clam are two examples of filter feeders. In the case of the clam, the surfaces of the gills are covered with cilia. The rhythmic beating of these cilia draws fresh water in through an opening, the incurrent siphon (Fig. 7.4). This water then passes through pores in the gills where it gives up oxygen to supply the respiratory needs of the clam. The gills are covered with a sticky mucus, and any solid particles in the water, including diatoms, bacteria, etc., are likely to be trapped in this mucus. Larger particles and indigestible particles drop fom the gills and are swept out the excurrent siphon by cilia lining the mantle. The mucus with its load of carefully screened particles is swept forward to the mouth while the now-filtered water passes out through the excurrent siphon. The parti-

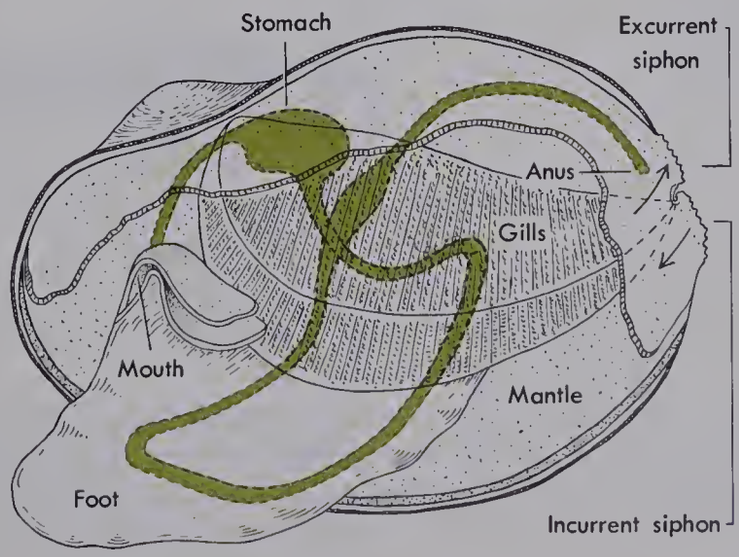


Fig. 7.4 The digestive apparatus of the clam. Food particles are filtered by the gills from the water drawn in through the incurrent siphon.

cle-laden mucus is then ingested. After digestion, any remaining material (the feces) is egested from the anus and passes out with the water flowing out the excurrent siphon.

Advantages of a tubular digestive system

The tubular digestive system of the clam is an improvement over the saclike system of flatworms because it permits a one-way flow of food materials and consequently greater efficiency in their handling. Incoming food need not mix with food that is almost completely digested or with wastes. The various stages in chemical digestion can follow one another in an assembly-line fashion. Each portion of the alimentary canal may be modified to carry out one, or a few, special aspects of the entire process. Along with the increased efficiency which a tubular alimentary canal permits, we find that in more complex animals, portions of the alimentary canal are pushed out into accessory structures, the digestive glands. These produce digestive enzymes and other important substances which then flow through ducts to the main portion of the alimentary canal. Here the foods are completely digested into a solution of molecules which can be absorbed by the cells lining the alimentary canal.

7.5 ACTIVE FOOD SEEKERS: THE GRASSHOPPER AND THE HONEYBEE

Most insects and most vertebrates are capable of rapid locomotion and thus are able to seek their food actively. The grasshopper moves from plant to plant by walking, hopping, and flying. Specialized appendages around the grasshopper's mouth enable it to consume large quantities of plant leaves quickly and efficiently (Fig. 7.5). The plant material passes from the mouth into the alimentary canal for digestion and absorption. First it passes through the esophagus and into a temporary storage organ, the crop (Fig. 7.5). From here it travels to the muscular gizzard. The gizzard is lined

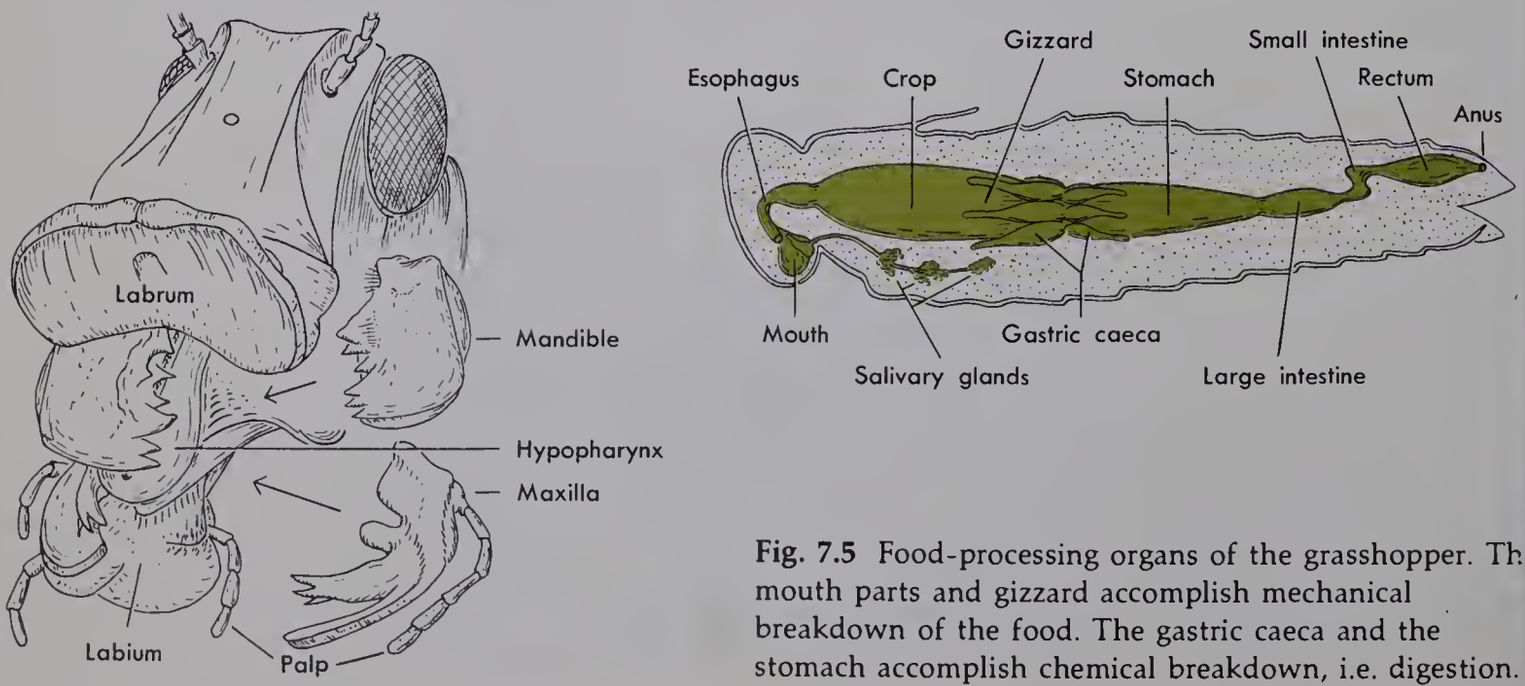


Fig. 7.5 Food-processing organs of the grasshopper. The mouth parts and gizzard accomplish mechanical breakdown of the food. The gastric caeca and the stomach accomplish chemical breakdown, i.e. digestion.

Animals that eat solid food generally break it up mechanically before digesting it

with rigid plates and the muscular action of the gizzard grinds the food material into still smaller particles. Efficient **mechanical breakdown** of food is thus one other advantage that a tubular alimentary canal can provide. The food then passes into the stomach where **chemical digestion** of it takes place. Digestive enzymes manufactured in six gastric glands (caeca) are secreted into the stomach to carry out this function. Some food may actually enter the caeca during the process. Digested food and a good deal of water are then absorbed in the intestine. The feces are stored temporarily in the rectum before being **egested**, relatively dry, through the anus.

The mouth of the honeybee

The mouth parts of the honeybee are quite different in appearance and adapted for manipulating a different kind of food. The adult honeybee feeds on liquid food, either the nectar of flowers or the honey stored within the hive. Her maxillae and labium are modified to form a tube through which this food is drawn up into the mouth by the pumping action of the tongue. The whole apparatus is called a **proboscis** (Fig. 7.6).

Most of the nectar ingested by a honeybee is destined to be taken back to the hive to be converted into honey. After passing down the long, thin esophagus, it is stored in the honey stomach (Fig. 7.6). The nectar is normally prevented from passing from the honey stomach to the real stomach by a valve located between the two. When the worker gets back to the hive, she regurgitates the nectar, and the process of converting it into honey begins.

The honeybee also uses her mouth to manipulate solids such as the wax out of which the hive is constructed. She uses her mandibles for this process and therefore it is not surprising that these mouth parts are rather like the mandibles of the grasshopper in their structure.

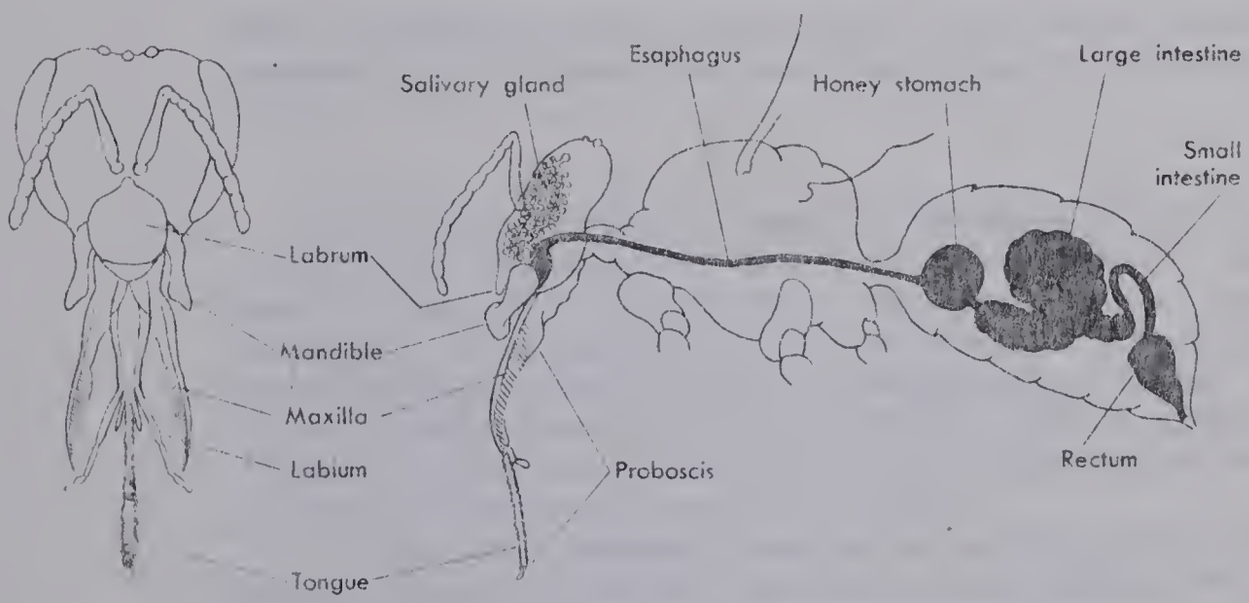


Fig. 7.6 Food-manipulating structures of the honeybee. The maxillae and labium form a proboscis with which the bee sucks up liquid food such as nectar. The honey stomach holds this food while it is being transported back to the hive.

7.6 INGESTION

The strategy by which the human animal digests its food is similar to that of the grasshopper. Once placed within the mouth, the food is ground into small bits by use of our teeth. As in the grasshopper, then, mechanical breakdown of food precedes chemical breakdown (digestion). This is important because it makes the food more easily swallowed and at the same time greatly increases the surface area that will be exposed to the action of digestive enzymes.

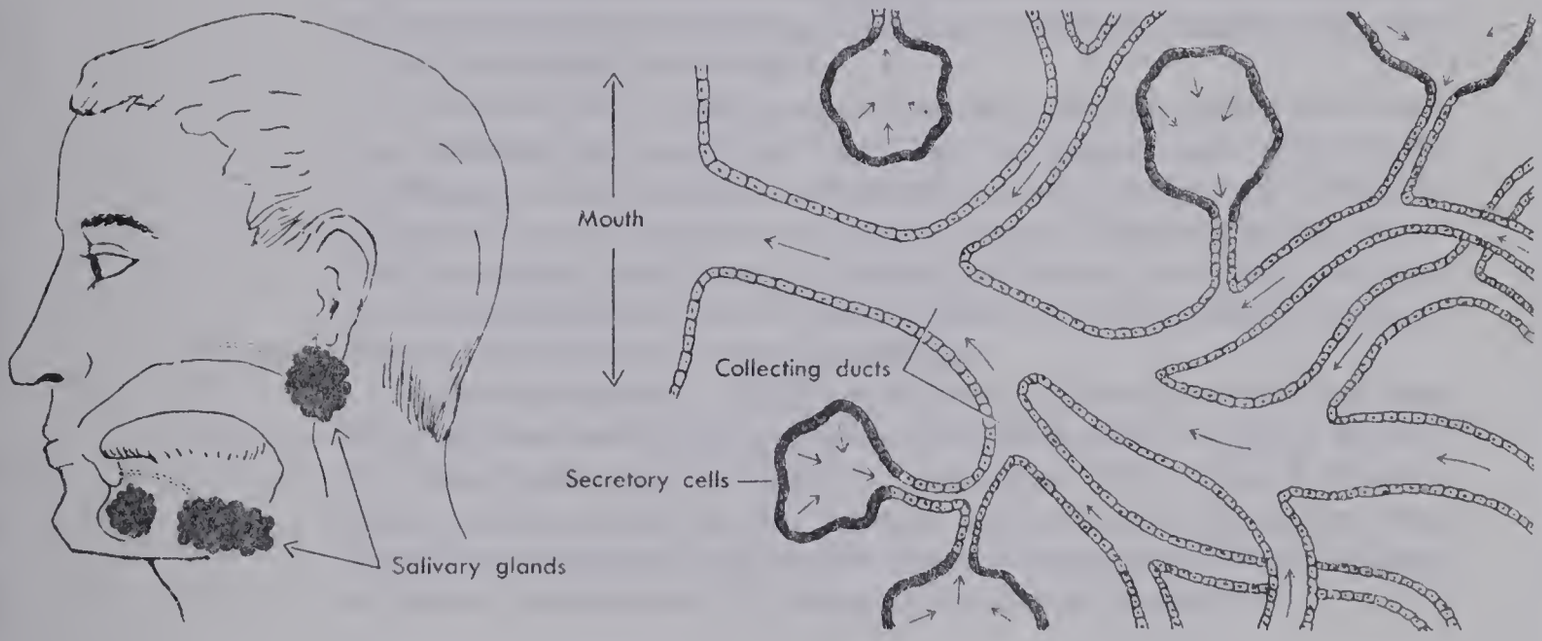


Fig. 7.7 Organization of human salivary glands. As in all exocrine glands, their secretions pass into a system of ducts which lead ultimately to the exterior of the body.

Saliva

While the food is within the mouth, it is moistened by saliva. Saliva is secreted into the mouth from three pairs of glands (Fig. 7.7) that are under the control of the nervous system. The sight, smell, taste, and even the thought of food can all trigger the passage of saliva from the glands through ducts into the mouth. Saliva is a somewhat sticky, "slippery" fluid because it contains a slimy carbohydrate-protein compound called mucin. The mucin enables the saliva to bind the small food particles together into a soft mass which can then be easily swallowed.

The saliva also contains an **amylase**, or starch-digesting enzyme, which catalyzes the hydrolysis of starch into the sugar maltose. This amylase is often called ptyalin, although under the rules for naming enzymes (see Section 6.8) the name salivary amylase is preferred. You can easily demonstrate its action by chewing an unsweetened cracker. After a short time, a distinctly sweetish taste will become noticeable.

Saliva is only the first of a number of secretions that flow into the alimentary canal and aid the process of digestion. In each case, these secretions are synthesized within accessory structures called glands. Ducts carry the secretions from the glands to the alimentary canal. The inner surface of each gland is continuous with the inner surface of its ducts (Fig. 7.7) and also with the inner surface of the alimentary canal. In fact, all these digestive glands are formed during embryonic development from outfoldings of the alimentary canal.

The ducts of all exocrine glands are really extensions of the external environment

When food is swallowed, it passes through the pharynx and into the esophagus. The esophagus is a thick-walled, muscular, straight tube leading from the pharynx to the stomach. It extends through the neck and the chest cavity. Its function can perhaps be best understood as a quick means of conveying food past the large, vital, but nondigestive organs of the chest cavity: the lungs and the heart.

The wall of the esophagus also contains glands that secrete mucin and thus lubricate the passage of food. Once the food mass is well into the esophagus, its movement is controlled by muscles in the wall of the esophagus that are not under our voluntary control. Those just in front of the food mass relax while those just behind it contract. As a result, the food mass is propelled downward. These rhythmic waves of muscular relaxation and contraction are called **peristalsis.**

Below the muscular diaphragm, which separates the chest cavity from the abdominal cavity, the esophagus joins the stomach. A ring of muscle, the cardiac sphincter, surrounds the esophagus at this point. It acts as a valve, relaxing to let into the stomach any food that approaches from above but remaining closed at other times. (The process of vomiting does, of course, also involve the opening of the cardiac sphincter.)

7.7 THE STOMACH

The stomach is a large pouch located high in the abdominal cavity (Fig. 7.8). Its wall is lined with millions of tiny gastric glands, which secrete 400–800 ml of gastric juice at each meal. The incoming food is thoroughly mixed with the gastric juice by vigorous muscular contractions of the stomach. (When these contractions occur in an empty stomach, hunger "pangs" result.)

There are several kinds of glands in the wall of the stomach. One secretes a dilute solution of hydrochloric acid. This gives the gastric juice a pH of about 1 to 2. Other glands secrete a solution containing the protein pepsinogen. In the presence of HCl, the pepsinogen is converted into the protein-digesting enzyme, **pepsin.** Pepsin hydrolyzes ingested proteins into peptide molecules by breaking the bonds between certain specific amino acid links in the chain. The peptide molecules produced may still contain dozens of amino acids.

Spallanzani first demonstrated that gastric juice digests meat

Our present knowledge of the digestive activity of the stomach has been gained from the research of many men. As early as 1776, the Italian scientist Spallanzani discovered that human gastric juice digests meat. He

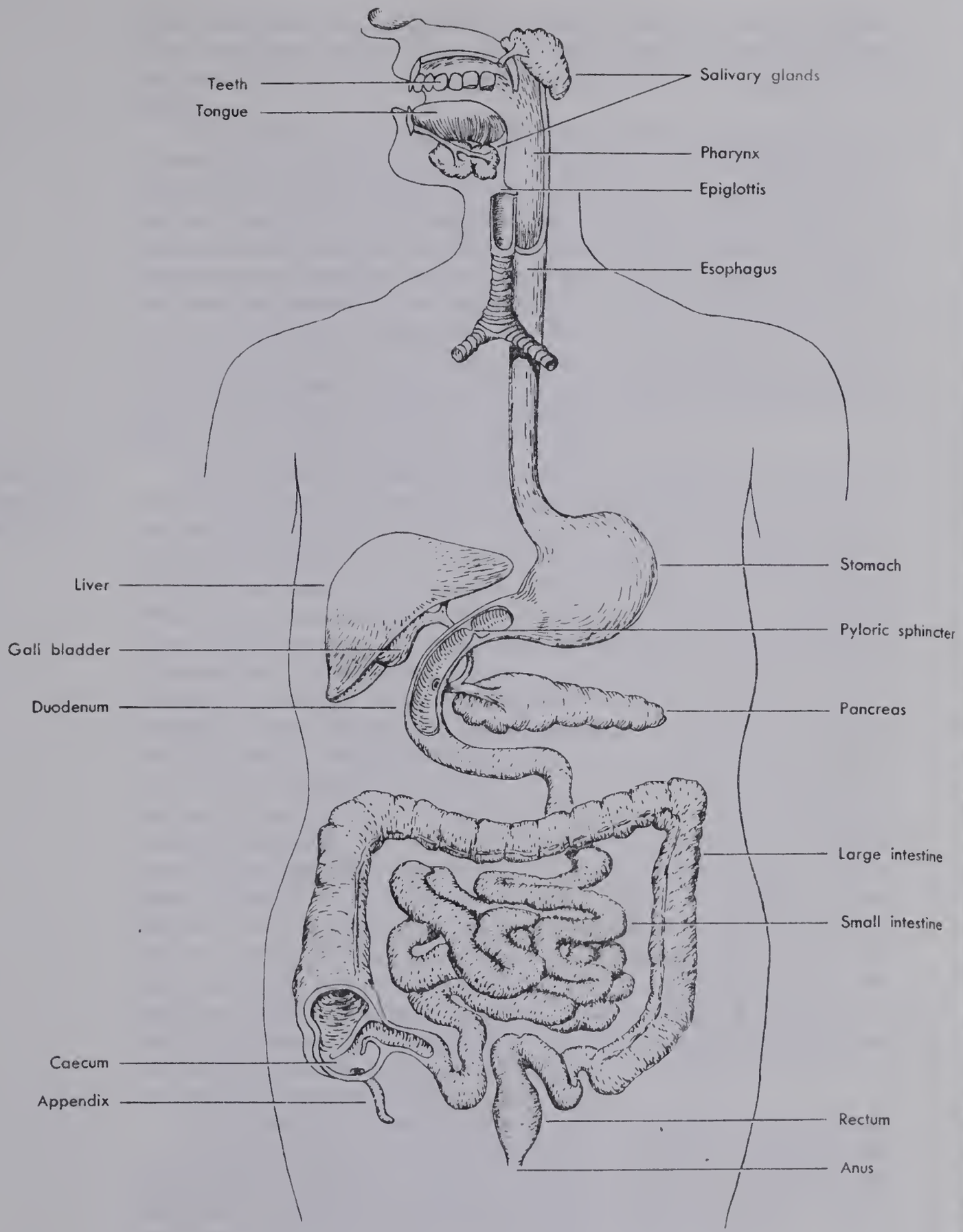


Fig. 7.8 Digestive system of the human.

built small cages of wire in each of which he placed a piece of sponge. After attaching a string, he would swallow a cage for a period of time and then retrieve it. The juice squeezed from the sponge digested meat readily.

The work of the American physician William Beaumont is also a landmark in the history of research on gastric activity. In 1822 he was called upon to treat a trapper, Alexis St. Martin, who had suffered a serious gunshot wound. The blast had penetrated both the muscular wall of the abdominal cavity and the wall of the stomach. Under Beaumont's care, St. Martin made an excellent recovery. However, the margin of the wound in the body wall healed to the margin of the wound in the stomach. As a result, a permanent opening into the stomach was formed. Beaumont recognized the research possibilities of this occurrence. For the next eight years (interrupted at intervals when St. Martin tired of his role) Beaumont supported St. Martin and his growing family while carrying on a classic series of investigations of his gastric activity.

Pavlov demonstrated that the secretion of gastric juice is partly controlled by a hormone

In the 1890's, the Russian physiologist Ivan Pavlov discovered the mechanisms controlling the release of gastric juice. He found that after he cut nerves leading to the stomach of dogs, the amount of gastric secretion was reduced by about 25%. This showed definite, but incomplete, control of gastric secretion by the nervous system. What about the remaining 75%? Pavlov reasoned that a hormone might be liberated in the blood when food enters the stomach, which would also stimulate the production of gastric juice. He tested this theory by connecting the blood circulatory systems of two dogs. When one dog was fed, it was only a short time before the second dog began to secrete gastric juice also. The second dog, however, secreted only about 75% of the normal amount. We know now that a hormone, **gastrin**, is produced by certain cells in the stomach when food enters the stomach. This hormone diffuses into the blood stream and is carried throughout the body. When blood containing this hormone returns to the stomach, it stimulates the gastric glands to secrete gastric juice. In Pavlov's experiment, the hormone also passed into the blood stream of the second dog, causing its gastric glands to start functioning, too. The joining of the circulatory systems of two animals in this way is called parabiosis. It is a very valuable tool for studying the action of the hormones, which are almost exclusively transported by the blood. The operation is always carried out with scrupulous care, under anesthesia, and every effort is taken to ensure that the animals suffer no unnecessary discomfort at any time during the procedure.

As the contents of the stomach become thoroughly liquefied, they pass through a sphincter at the lower end of the stomach, and into the small intestine, the first ten inches of which is called the **duodenum**. An opening in the wall of the duodenum leads to two ducts which, in turn, drain two large digestive glands, the pancreas and the liver.

7.8 THE PANCREAS

Pancreatic fluid drains into the duodenum

The pancreas is an elongated, whitish gland which lies in the loop formed by the duodenum and the under surface of the stomach. Secretory cells in the pancreas produce pancreatic fluid which then passes down the pancreatic duct and into the duodenum. The pancreatic fluid contains the following substances.

a) **Sodium bicarbonate** ($NaHCO_3$). The sodium bicarbonate neutralizes the acidity of the intestinal contents, quickly raising the pH to about 8.

b) An **amylase.** This pancreatic amylase hydrolyzes starch into maltose Because it has a much longer period in which to act, it plays a more important role in starch digestion than does salivary amylase.

Bile facilitates fat digestion

c) A **lipase.** The pancreatic lipase hydrolyzes about one-third of the ingested fats into glycerol and fatty acid molecules. Its action is enhanced by a secretion of the liver called **bile.** Bile contains substances (steroids) called bile salts, which emulsify fats. Large drops of fat (oil) are broken into tiny droplets by the detergent action of these bile salts. This action increases the surface area exposed to the pancreatic lipase and thus hastens fat digestion.

d) **Two proteinases.** Trypsin and chymotrypsin are both enzymes that continue the work of protein digestion by hydrolyzing specific peptide bonds that were not attacked by pepsin. (The action of pepsin ceases when the sodium bicarbonate raises the pH of the intestinal contents.) Trypsin and chymotrypsin work most rapidly at the higher pH (Fig. 7.9). Like pepsin, both trypsin and chymotrypsin are secreted in an inactive form and only when within the duodenum are they converted into their active state

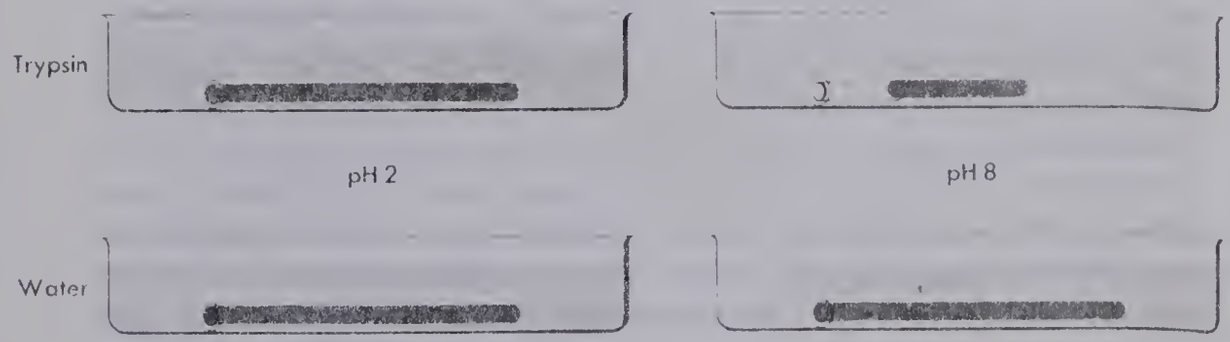


Fig. 7.9 The effect of pH on the digestive action of trypsin. The contents of each bottom dish are the same as those of the one above it except that no enzyme is present. Thus the bottom dishes serve as "controls" for the experiment. The glass tubes contain cooked egg white.

e) **Carboxypeptidase.** This enzyme removes, one by one, the amino acids located at the acid end of peptide molecules (Fig. 7.10). Thus it aids in the hydrolysis of peptides into amino acids.

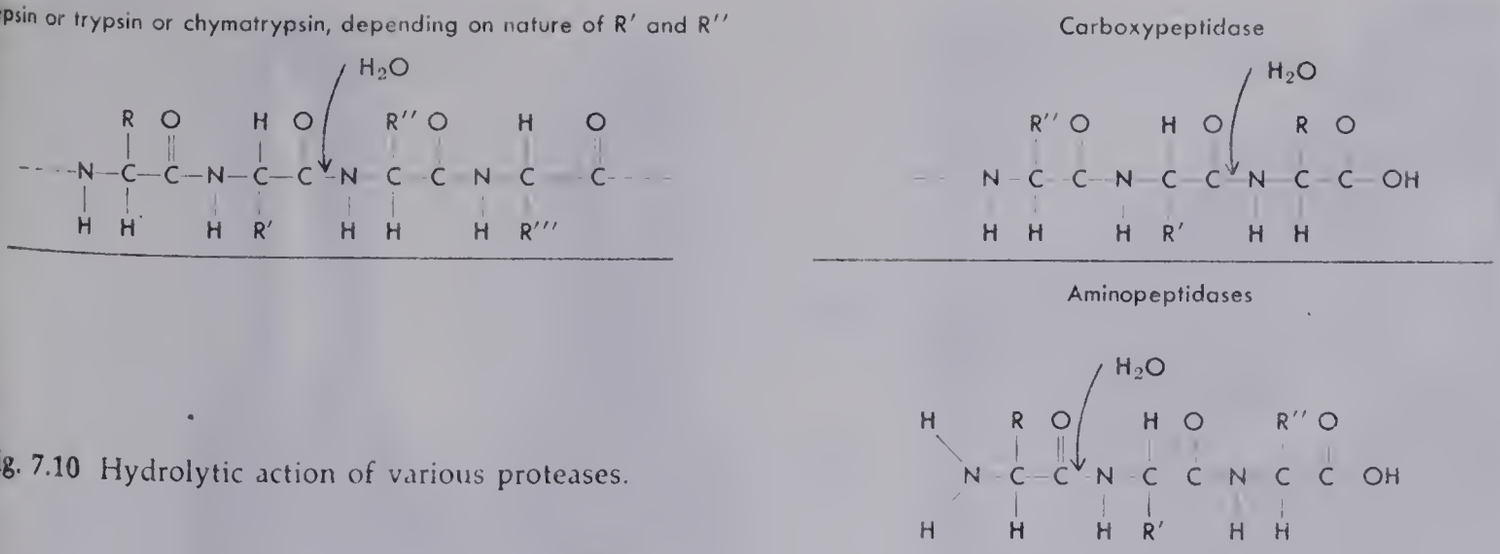


Fig. 7.10 Hydrolytic action of various proteases.

f) **Nucleases.** These enzymes hydrolyze ingested nucleic acids (RNA and DNA) into their component nucleotides.

The secretion of pancreatic juice is under hormonal control. When the acidified contents of the stomach enter the duodenum, certain cells in the wall of the duodenum release the hormones secretin and pancreozymin into the bloodstream. When carried to the pancreas, these stimulate the production and discharge of pancreatic fluid. Secretin chiefly affects the secretion of sodium bicarbonate solution whereas pancreozymin stimulates the production of the digestive enzymes.

7.9 THE SMALL INTESTINE

As the mixture of food, bile, and pancreatic enzymes passes through the small intestine, maltose, peptides, fatty acids, glycerol, and fat droplets are produced. The final digestion and absorption of these materials is the function of the **villi,** which line the inner surface of the small intestine (Fig. 7.11). The villi increase the surface area of the small intestine many times what it would be if it were simply a tube with smooth walls. In addition, the exposed surface of the epithelial cells of each villus is covered with projections, the **microvilli** or "brush border." Thanks largely to these, the total surface area in the intestine is about 100 ft^2, five times the surface area of the exterior of the body.

The villi

Incorporated in the surface of the microvilli are a number of enzymes that complete the digestion process. Several **aminopeptidases** complete the hydrolysis of peptides into amino acids. These enzymes act in a manner similar to carboxypeptidase but attack the amino ($—NH_2$) end of the peptide molecules instead of the acid end (Fig. 7.10).

Several digestive enzymes are incorporated in the membranes of the microvilli

Three **disaccharidases** localized on or in the microvilli hydrolyze the disaccharides maltose, sucrose, and lactose into their component monosaccharides. Each maltose molecule is split into two glucose molecules and thus glucose is the end-product of the digestion of starch. Sucrose (com-

Anabolism

Catabolism

Fig. 8.1 Pathway
"pool" of organic
molecules can be
they can be used
macromolecules w

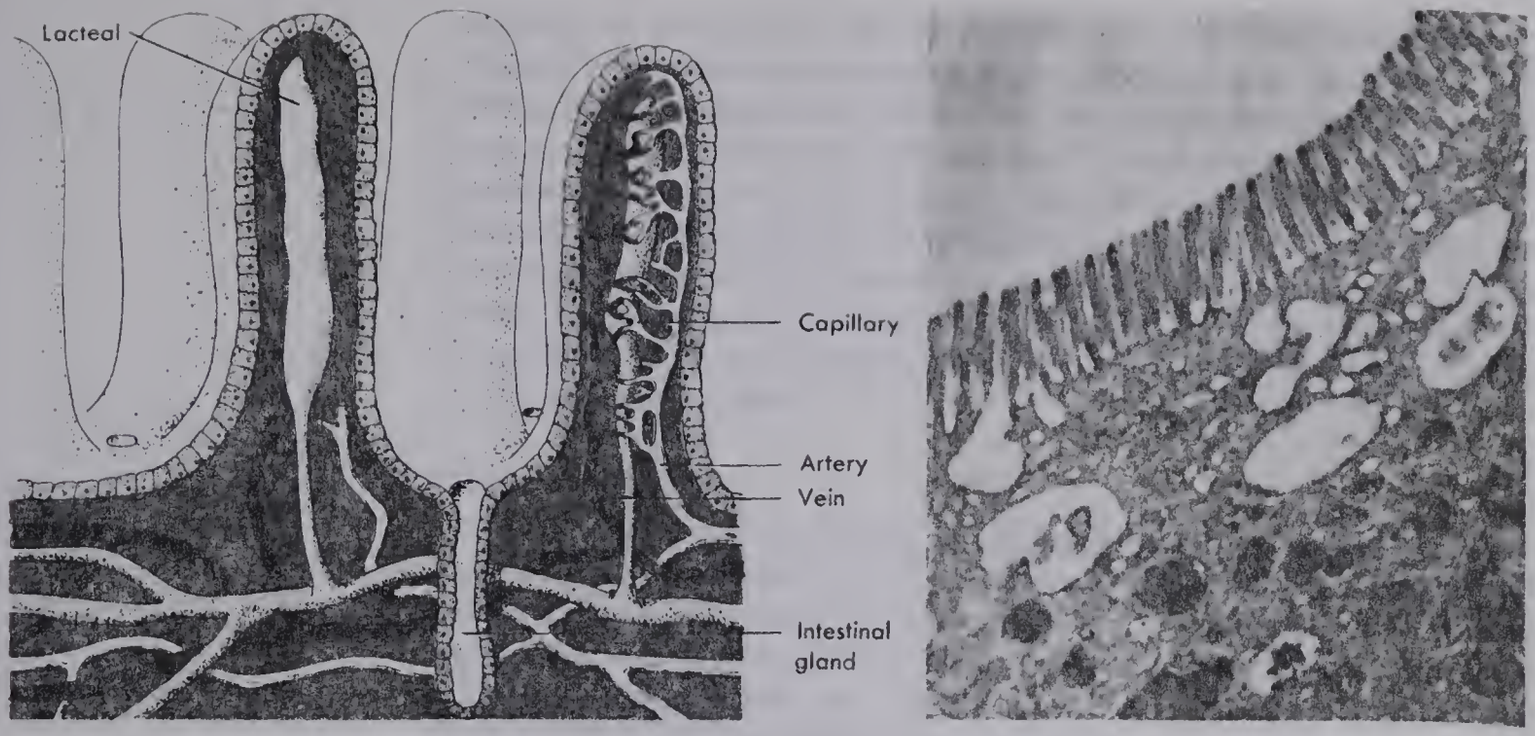


Fig. 7.11 Villi. Left: Digested foods enter the capillaries. Oil droplets enter the lacteals. The villi, with their microvilli, provide approximately 100 square feet of absorbing area in the small intestine. Right: electron micrograph (8000X) of the microvilli of mouse intestine cells. (Courtesy of Dr. Sam L. Clark, Jr. Reprinted by permission from The Rockefeller Institute Press, *Journal of Biophysical and Biochemical Cytology* 5, 41–50, 1959.)

mon table sugar) gives a molecule of glucose and one of its isomers, fructose, while lactose (milk sugar) gives glucose and galactose (Fig. 4.2).

With the action of these enzymes, protein and carbohydrate digestion comes to an end. What were originally macromolecules have now been converted into small molecules (amino acids and monosaccharides) ready for passage into the bloodstream.

The absorption of food

Each villus is richly supplied with blood which passes through a network of tiny tubes called capillaries. The sugars and amino acids as well as vitamins, salts and some water pass from the intestinal contents into the capillaries of the villus. Although this transport may be accomplished by diffusion in some cases, other mechanisms are involved, too. Glucose, for example, continues to be absorbed even when its concentration in the intestinal fluid becomes less than its concentration in the blood (about 0.1%). This absorption is accomplished by active transport.

Endocytosis may also play a significant role in the absorption of digested foods. You remember that only about one-third of the ingested fats becomes hydrolyzed to fatty acids and glycerol. These molecules, acting with the bile salts, emulsify the remaining two-thirds into tiny droplets. The droplets are then absorbed, most likely by endocytosis, into the epithelial cells of the villus. Once inside, many of the fatty acid and glycerol molecules become resynthesized into fat in the smooth endoplasmic



reticulum of these cells. The fat droplets are then discharged into the interior of the villus by exocytosis. These droplets do not enter the capillary network. Instead they enter a duct, the lacteal, whose walls are more porous. The lacteals of the villi are part of the lymphatic system. The morphology and physiology of this system will be discussed in Chapter 13. Once inside the lacteals, the fat droplets are carried slowly through the lymphatic system until it joins with the regular blood circulatory system. After a fat-rich meal, the lacteals take on a whitish, milky appearance (*lac* is the Latin word for milk) because of the large numbers of fat droplets contained within them.

7.10 THE LIVER

The liver secretes bile

Although the liver is not strictly an organ of digestion, its secretion, bile, does play an important role in the digestion of fats. Bile is produced continuously by the liver but between meals it accumulates in a storage organ, the gall bladder. When food enters the duodenum, a hormone (cholecystokinin) is released to the bloodstream from cells in the lining of the duodenum. Upon reaching the gall bladder, this hormone stimulates it to contract and expel its contents of bile into the duodenum. The action of this hormone, as well as the action of gastrin, secretin, and pancreozymin, illustrates nicely an important fact about hormonal control. In each case, the liberated hormone is carried by the blood to every organ, every tissue, every cell of the body. Only certain organs, however, are capable of responding to the presence of the hormone. These are called the target organs.

Bile contains other materials besides bile salts. Among these are the bile pigments. These are the products of the breakdown of the red blood pigment, hemoglobin, removed by the liver from old red blood cells. The brownish color of the bile pigments imparts the characteristic brown color of the feces.

The formation of bile is just one of several important nutritional functions carried out by the liver. Before the blood that leaves the villi reaches the general circulation, it passes through the liver. Here extraordinary components (e.g. nonnutritive molecules) that are picked up by absorption from the intestine, or excess amounts of ordinary components, are screened out. All monosaccharides except glucose (e.g. fructose, galactose) are removed by the liver and converted into glucose. Any glucose in excess of the normal 0.1% concentration found in the blood is removed and converted into the insoluble polysaccharide, glycogen.

Urea is synthesized in the liver

Amino acids in excess of the body's anabolic needs are deaminated by the liver. In deamination, the nitrogen-containing amino ($-NH_2$) portion of the molecule is removed. This is then converted into a nitrogenous waste, urea. The mechanism of this process will be discussed in Chapter 15. The non-nitrogen-containing residue of the amino acid can then enter the metabolic pathway of cellular respiration and be oxidized for energy. The details of this mechanism will be considered in the next chapter.

The hepatic portal system

The system of blood vessels that carries all blood received from the intestines through the liver before passing it to the general circulation is called the **hepatic portal** system. In a real sense, this system provides a gatekeeper between the intestines and the general circulation. In this role, the liver screens the blood passing through it so that the composition of the blood when it leaves will be that which is normal for the organism. For example, even after a meal rich in starches and sugars, the glucose level of the blood leaving the liver will not be much higher than the normal 0.1%.

The liver adjusts the composition of the blood with respect to a number of organic substances

On the other hand, if the concentration of glucose in the blood should start to fall between meals, the liver will hydrolyze enough of its glycogen reserves to maintain the level at 0.1%. In other words, the liver is one of the most important devices in the human body for maintaining the constancy of the ECF and thus for the preservation of homeostasis. In Chapter 25 the liver's role in maintaining homeostasis with respect to the sugar content of the ECF will be explored further.

7.11 THE LARGE INTESTINE

The small intestine leads into the large intestine. A sphincter controls the passage of materials from one to the other. Just beyond this sphincter is a blind pocket, the caecum. Attached to the caecum is a tiny projection, the appendix. It is considered to be the vestigial remains of a structure which may have functioned in cellulose digestion in some very remote, prehuman ancestor. Its chief interest to man is the fact that it may become infected, causing appendicitis. A severe infection may cause the appendix to rupture, thus spreading the infection to the membranes lining the abdominal cavity and supporting the organs (the viscera) within it. This condition is known as peritonitis.

The large intestine receives the liquid residue of material left after digestion and absorption in the small intestine have been completed. This residue contains substantial quantities of water and indigestible substances such as cellulose. Some other food materials are invariably present and these serve to nourish the enormous population of bacteria that live in the large intestine. Usually these bacteria (of which the most common species is the much-studied *Escherichia coli*) are perfectly harmless. In the process of their metabolic activities they may produce gases and other odoriferous wastes. They flourish to such an extent that from 10% to 50% of the dry weight of the feces may consist of bacterial cells.

An enormous population of bacteria lives in the large intestine

Occasionally, harmful bacteria or protozoans may become established in the large intestine. Typhoid fever, asiatic cholera, and amoebic dysentery are three diseases which are caused by intestinal parasites. These parasites may even invade other organs of the body such as the liver and cause extensive damage.

The large intestine reclaims water from its contents

The chief function of the large intestine is the reabsorption of water. A great deal of water is secreted into the stomach and intestine by the various digestive glands. This must be reclaimed if the individual is not to suffer from dehydration and thirst. Most of the water is reabsorbed in the

large intestine during the 12 to 14 hours that the food residues remain there. Sometimes the large intestine becomes irritated and discharges its contents into the rectum and out of the anus before water reabsorption is complete. The resulting condition is called diarrhea. Perhaps you have noticed the thirst which accompanies a case of diarrhea. On the other hand, the large intestine may retain its contents for an extra long period. The fecal material, as it is now called, becomes dried out and compressed into dry, solid masses. This condition is known as constipation.

The feces pass from the large intestine through an S-shaped tube into the rectum. Here they remain until the two sphincters guarding the anus relax and vigorous peristaltic waves expel them in the process of defecation.

EXERCISES AND PROBLEMS

1. Outline the digestion of a bacon (protein and fat) sandwich (starch).
2. Aquatic vertebrates such as fresh-water fishes and frogs have little or no large intestine. Can you think of an explanation for this?
3. By what mechanisms might amino acids in the small intestine enter the epithelial cells of the villi?
4. Is the digestion of macromolecules a process that occurs only in the digestive tract? Explain.
5. How does chewing or otherwise breaking food down mechanically aid digestion?
6. How can the amoeba digest the food it engulfs by endocytosis without digesting itself?
7. How do cells that secrete extracellular enzymes avoid self-digestion?
8. What is sucrase?
9. Do any filter-feeders live on land? Explain.

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4. Kretchmer, N., "Lactose and Lactase," *Scientific American,* Offprint No. 1259, October, 1972. In many human populations, the adults lack lactase and cannot digest milk.

8

ENERGY PATHWAYS IN THE CELL

Fermentation

Cellular Respiration

Anaerobic Breakdown

The Cytochrome Enzyme System

The Citric Acid Cycle

Importance of Cellular Respiration

How These Discoveries were Made

The Uses of Energy

In the last chapter we examined some of the mechanisms by which heterotrophic organisms *ingest* large organic molecules and then *digest* these into their subunits. Very little energy is liberated in this process of hydrolysis. Most of the chemical energy stored in starches, proteins, and fats is still locked up in the end products of their digestion: sugars, amino acids, fatty acids, and glycerol. The process of digestion does accomplish a marked reduction in the size of the molecules, so that they may be absorbed readily from the ECF into the cytoplasm of cells.

What is the fate of these small, organic molecules once they enter the cytoplasm? Most of them are further reduced in size, forming still simpler molecules containing, characteristically, two to four carbon atoms. These small molecules then face one of two alternatives. They may proceed back "up" various metabolic pathways and serve as the building blocks of sugars, fatty acids, glycerol, and amino acids. From these can then be assembled the macromolecular components of the cell: polysaccharides, lipids, proteins, and even nucleic acids (Fig. 8.1). This phase of metabolism, in which large, complex molecules are built up from smaller, simpler ones, is called **anabolism.**

Anabolism

The second fate of the intracellular pool of 2- to 4-carbon molecules is to be still further degraded—ultimately to simple, inorganic molecules like CO_2, H_2O, and NH_3 (Fig. 8.1). The total amount of energy stored in the end products of these decompositions is much less than the amount of energy present in the original molecules. Thus, at most of the degradative steps, energy is released. This phase of metabolism, in which relatively complex, energy-rich molecules are broken down into simpler, energy-poor molecules, is called **catabolism.** Living organisms accomplish the breakdown by two different (but related) processes: fermentation and cellular respiration.

Catabolism

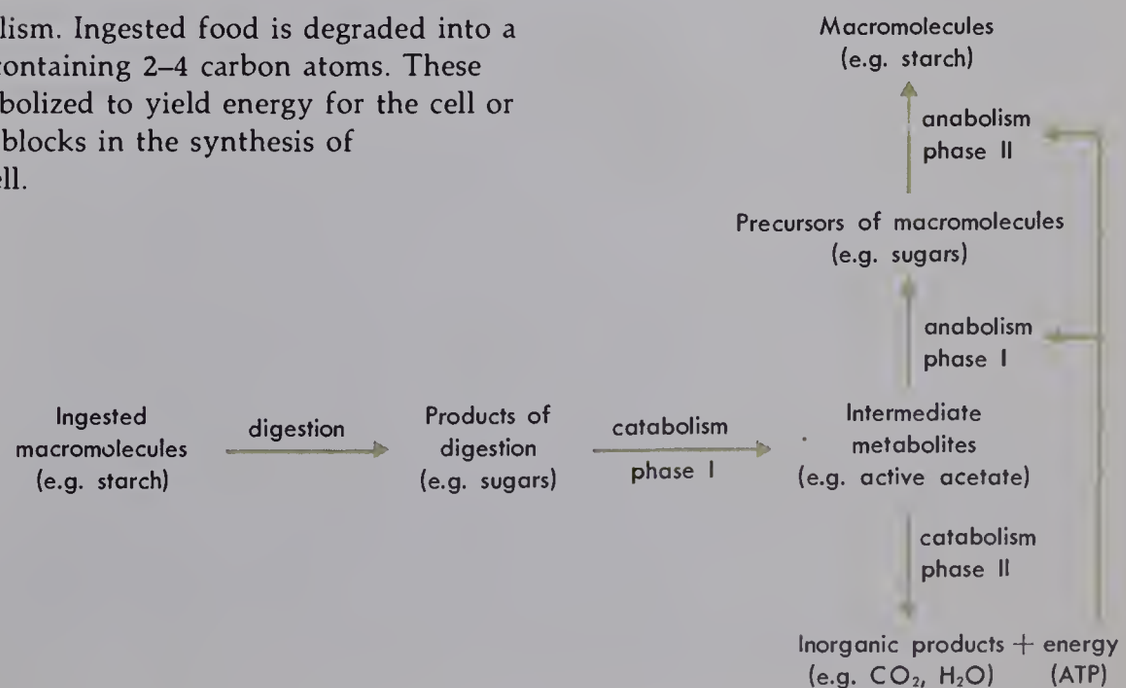


Fig. 8.1 Pathways of metabolism. Ingested food is degraded into a "pool" of organic molecules containing 2–4 carbon atoms. These molecules can be further catabolized to yield energy for the cell or they can be used as building blocks in the synthesis of macromolecules within the cell.

8.1 FERMENTATION

One method of securing energy from complex organic molecules is to fragment them into simpler molecules. An example of this process is the chemical breakdown of glucose into **ethyl alcohol** and **carbon dioxide.** This process is called alcoholic fermentation. It may be represented by the equation:

Alcoholic fermentation

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2.$$

Inspection of this equation shows that glucose is the only starting material. No other substance, not even oxygen, is required. Because oxygen is not required, the process is **anaerobic;** that is, it can go on in the absence of air. Every atom originally present in the glucose is accounted for in the products. The total energy stored in the products is, however, somewhat less than that stored in the glucose molecule. The energy difference is liberated in the process.

Anaerobic means "in the absence of molecular oxygen"

Although the process of alcoholic fermentation has probably been used by man through most of human history, it was not until about 100 years ago that scientists began to understand its nature. The great French biochemist Louis Pasteur found that alcoholic fermentation was always associated with the presence and growth of single-celled fungi, the yeasts. The converse was not true, however. He found that yeasts when exposed to adequate amounts of air produced mostly H_2O and CO_2 as the end products of their metabolism. Pasteur concluded that fermentation was an energy-producing mechanism used by organisms deprived of air. He called fermentation "life without air." Since Pasteur's time, the enzymes and coenzymes that carry out the chemical changes in alcoholic fermentation have been isolated and the details of the process determined. Because of the close relationship between these intermediate steps and those involved in the process of cellular respiration, we will defer a discussion of them until that topic is considered (see Section 8.3).

Pasteur called fermentation "life without air"

Muscles ferment glucose to lactic acid

Other kinds of fermentation are also known. For example, when vigorous activity of our muscles outstrips the supply of oxygen available for cellular respiration, the muscles get energy from the fermentation of glucose. (The glucose is, in turn, derived from animal starch, glycogen, which is stored in muscles.) Fortunately for us, the end product of this fermentation is **lactic acid,** rather than alcohol and CO_2.

Efficiency of fermentation

Both alcoholic and lactic acid fermentation are inefficient processes. Although the total energy in the products of fermentation is less than that in glucose, it is not much less. Not more than 6% of the energy stored in glucose is made available to the organism during fermentation. Most of the energy originally stored in the glucose molecule is still stored in the end products of ethyl alcohol or lactic acid. (The use of ethyl alcohol as a fuel component in some racing cars and rocket engines should make this clear.)

Not only is fermentation an inefficient method for securing energy, but it is potentially a hazardous one as well. Ethyl alcohol is poisonous in moderate concentrations. The activity of yeast plants ceases when the

alcoholic concentration of the medium in which they are growing reaches about 14%. (This sets a limit on the alcohol concentration of undistilled alcoholic beverages such as natural wines.) The accumulation of excessive amounts of lactic acid in human muscles is undesirable because it lowers the pH of the cell contents and ECF. Too high a concentration of lactic acid causes the muscle to become incapable of further action.

Fermentation is a wasteful process. Only a small fraction of the energy stored in the glucose molecule is made available to the organism. Most of it is still retained in the end products of fermentation. Therefore, organisms that rely on fermentation must have large amounts of glucose available to them in order to satisfy their needs. If, however, glucose could be broken down by cells into truly simple, energy-poor molecules (e.g. H_2O and CO_2), a much higher percentage of its energy would be released. Such a breakdown occurs when glucose is oxidized. Oxidation of glucose takes place within the cells of the vast majority of living organisms in the process of cellular respiration. It is carried out in the mitochondria (Section 5.9).

8.2 CELLULAR RESPIRATION

Cellular respiration can be defined as the oxidation of organic compounds that occurs within cells. In Chapter 3, we defined oxidation as the process of removing electrons from a substance. In cells, the most common type of oxidation involves the removal of hydrogen atoms (each with its electron) from the substance. The primary oxidizing agents in cells are the coenzymes nicotinamide-adenine dinucleotide, or **NAD,** and its close relative, nicotinamide-adenine dinucleotide phosphate (NADP). (These two substances have been known by a variety of other names of which DPN, for NAD, and TPN, for NADP, are still quite common.) Each of these is a complex molecule made up of (1) nicotinamide (a B vitamin), (2) two five-carbon ribose units, (3) the purine adenine, and (4) two (in NAD) or three (in NADP) phosphate groups (Fig. 8.2). Both of these substances can remove two electrons (usually associated with two hydrogen atoms) from certain organic compounds, thus oxidizing them. The two electrons actually unite with the NAD (or NADP), thus reducing it. You remember that in Section 3.9 we pointed out that the process of reduction is always inseparable from the process of oxidation. An oxidizing agent (e.g. NAD) is reduced by the substance it oxidizes. Because NAD and NADP are so similar in activity, we will refer to either simply as NAD. The reduced form will be indicated as $NADH_2$.

NAD and NADP are the coenzymes for many redox reactions in the cell

The over-all chemistry of cellular respiration has been known for many years. A major fuel in most living things is the sugar glucose. With the aid of oxygen taken in from the ECF, cells oxidize glucose to the compounds carbon dioxide and water. The reaction can be expressed by the equation:

The respiration of glucose

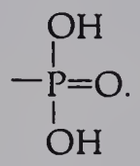
$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O.$$

Knowing what goes in and what comes out of a process is only part of the story, though. When ignited by a flame, glucose combines directly with oxygen, forming CO_2 and H_2O according to the equation just given. In living cells, however, the process proceeds in a more orderly and controlled fashion. The gradual discovery of the way in which glucose in living cells is oxidized to CO_2 and H_2O has been one of the great achievements of biochemistry.

8.3 ANAEROBIC BREAKDOWN

Activation of glucose by ATP

Glucose molecules must be activated by heating before they will begin to burn. In cells, also, glucose molecules must be activated before they can be respired. The activation is not accomplished by heat, but instead the energy of the molecule is increased by joining to it a phosphate group,



This process is called phosphorylation. Like all chemical reactions in cells, it requires an enzyme. It also requires a source of phosphate. The phosphate is supplied by a substance called adenosine triphosphate, or **ATP** for short. ATP consists of the purine adenine (what other substances contain this molecule?), ribose, and three phosphate groups (Fig. 8.3). The bonds by which the second and third phosphate groups are attached are known as "high-energy" bonds because of the large amount of energy released when these bonds are broken by hydrolysis. (They are shown in Fig. 8.3

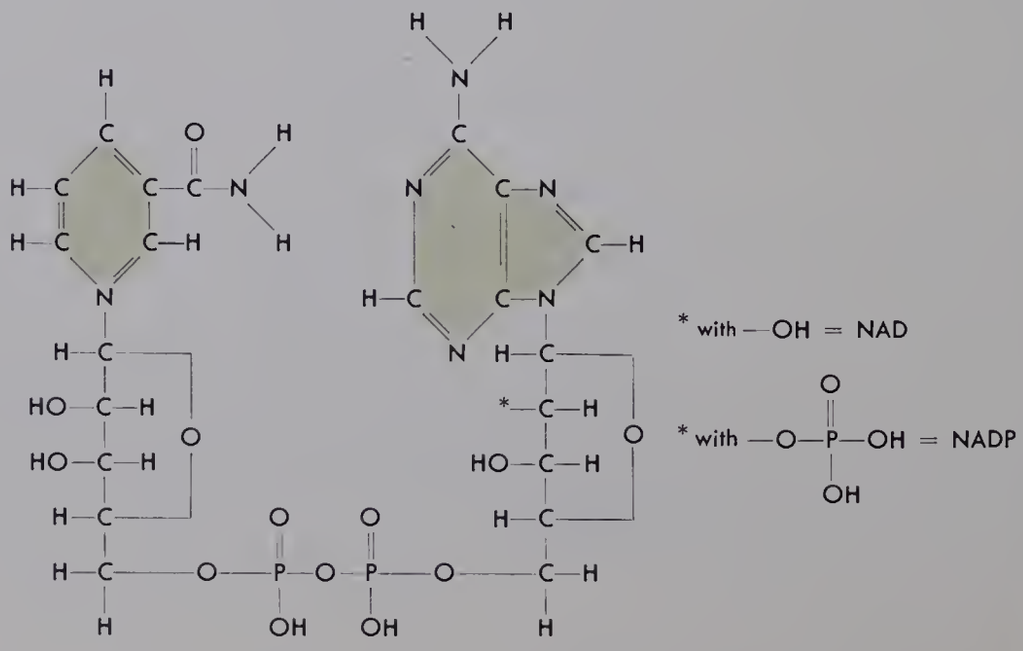
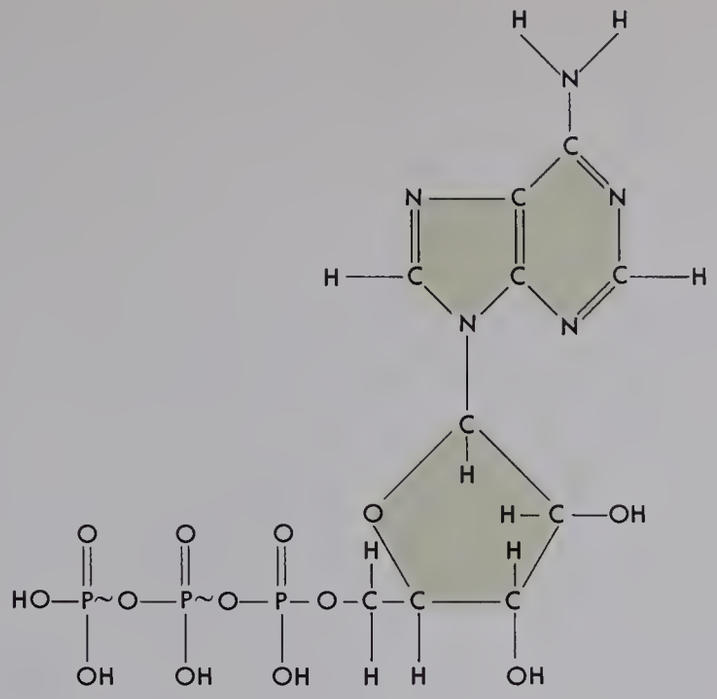


Fig. 8.2 Structural formula of nicotinamide adenine dinucleotide (NAD) and its phosphate (NADP).

Fig. 8.3 Structural formula of ATP. How does it compare with Fig. 4.14?



as wavy lines.) When glucose is phosphorylated, ATP transfers its third phosphate group and the energy of its bond to the glucose molecule. This results in an activated form of glucose, glucose-phosphate. In the same process, the ATP is changed to adenosine *di*phosphate (only two phosphates are left) or **ADP.** A minor enzyme-catalyzed rearrangement of the glucose molecule and the addition of one more phosphate group from a second molecule of ATP results in the formation of fructose-diphosphate. (Fructose is an isomer of glucose; i.e. it has the same molecular formula but a different structural formula.)

The fructose-diphosphate is then split into two halves. The resulting molecules, each containing three carbon atoms, are molecules of phosphoglyceraldehyde or **PGAL.** From this point on, we shall trace the path taken by just one molecule of PGAL. Bear in mind, however, that two molecules of PGAL have been produced from the original glucose molecule and hence all reactants and products involved from this point on are actually doubled in the process of respiring a single glucose molecule.

PGAL is oxidized to PGA

PGAL is then oxidized, as two electrons (and their hydrogen nuclei) are removed by NAD. A second phosphate group (donated by a molecule of the inorganic acid phosphoric acid, H_3PO_4) is added and immediately removed and transferred to a molecule of ADP to form ATP. The compound that results from these reactions is phosphoglyceric acid or **PGA** (Fig. 8.4). Two more chemical transformations result in the formation of a second molecule of ATP. With all the phosphate groups removed, a molecule of **pyruvic acid** ($C_3H_4O_3$) remains.

The conversion of glucose to pyruvic acid yields a small net gain of ATP

Note that the conversion of glucose to pyruvic acid does not require the presence of oxygen. It is an anaerobic process. Note also that although energy in the form of two ATP molecules is needed to "prime" the process, four ATP molecules (two from each of the PGAL molecules) are produced.



Fig. 8.4 The pathway of cellular respiration. When PGAL is oxidized by NAD, which occurs outside mitochondria, one step in ATP production (marked with an asterisk) is bypassed.

There is thus a net production of two molecules of ATP for each glucose molecule used. The total amount of energy stored in two pyruvic acid molecules is less than that stored in the glucose molecule. Some of the difference has been stored in the two molecules of ATP. Some of it is stored in the $NADH_2$. The remainder is liberated as heat.

The reduction of pyruvic acid to lactic acid

The energy stored in $NADH_2$ may be used directly by some energy-consuming activity of the cell. One example is the reduction of pyruvic acid to lactic acid:

$$C_3H_4O_3 \xrightarrow[NADH_2 \quad NAD]{} C_3H_6O_3.$$

This reaction is carried on by some bacteria and is also the final step in muscle fermentation (Section 8.1). Remember that the breakdown of glucose to pyruvic acid is an anaerobic process.

Careful biochemical studies have shown that the anaerobic breakdown of glucose to pyruvic acid proceeds in basically the same step-by-step fashion in both cellular respiration and muscle fermentation. In muscle fermentation, however, the $NADH_2$ formed by the oxidation of PGAL is used up in the reduction of pyruvic acid to lactic acid. There is, however, a net yield of two ATP molecules for every glucose molecule fermented, and it is this net yield which permits the muscle to continue to function for a time even when oxygen is not available.

Yeast cells convert pyruvic acid to ethanol and CO_2

Alcoholic fermentation also proceeds by the same biochemical pathway that we have been discussing. In alcoholic fermentation, however, a carbon dioxide molecule is removed from the pyruvic acid before reduction by $NADH_2$ occurs. This reaction thus yields a molecule of CO_2 and a molecule of ethyl alcohol:

$$C_3H_4O_3 \xrightarrow[NADH_2 \quad NAD]{} C_2H_5OH + CO_2.$$

Again, the organism (e.g. yeast) must satisfy all its energy needs from the net production of two ATP molecules for each glucose molecule consumed. Is it any wonder that yeast cells deprived of air grow relatively slowly and consume large amounts of glucose?

Every step in the anaerobic breakdown of sugar—whether in muscles, yeast cells, or as the first steps in cellular respiration—requires the catalytic action of a specific enzyme. All the enzymes participating in this anaerobic process are present in the cytoplasm of the cell, but they have not been found to be localized in any specific cell structures. We shall see, however, that all the remaining steps in the oxidation of sugar are accomplished by enzymes in the mitochondria.

8.4 THE CYTOCHROME ENZYME SYSTEM

In respiring cells, the usual fate of the $NADH_2$ is to be oxidized in turn by another coenzyme, a yellow substance called a flavin (Fig. 8.4). This oxidation, in some way as yet unknown, is *usually* coupled to the production of one ATP molecule from ADP and inorganic phosphate. The electrons received by the flavin are in turn passed on to an enzyme called cytochrome *b*, thus reducing it. Cytochrome *b* is, in turn, oxidized by

ATP molecules are generated as electrons pass through the cytochrome enzyme system

cytochrome *c*. This oxidation produces still another molecule of ATP. The electrons then pass to cytochrome *a* and then to the enzyme cytochrome oxidase. This last transfer results in the formation of a third ATP molecule. All these cytochrome enzymes are proteins with iron-containing prosthetic groups (Fig. 8.5). Iron, you remember (see Section 3.9), has a variable valence and thus is admirably suited to serve in redox systems. The prosthetic groups of the cytochrome enzymes are also quite similar to the prosthetic group, heme, of the red, oxygen-carrying pigment, hemoglobin, found in vertebrate blood. They also resemble the green plant pigment, chlorophyll, although the chlorophyll molecule contains an atom of magnesium rather than iron.

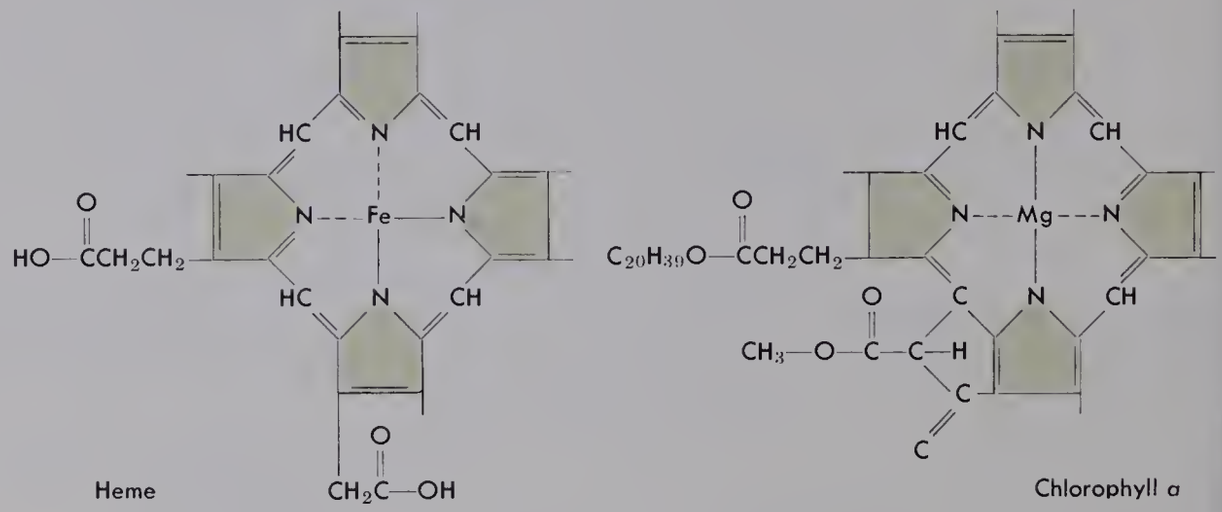


Fig. 8.5 Simplified structural formulas of heme and chlorophyll *a*.

Oxygen is the final electron acceptor in cellular respiration

Cytochrome oxidase donates its two electrons to an atom of oxygen which also picks up two H^+ ions to form a molecule of water. The ultimate electron acceptor then is oxygen just as it is when glucose is burned after being ignited by a flame. In the cell, however, the passage of electrons from the glucose to the oxygen proceeds in such small, separate steps, that almost 40% of the energy released in the oxidation can be conserved as chemical energy stored in ATP, rather than being liberated as heat.

One might well expect that a system requiring the efficient passage of electrons through a whole sequence of enzymes would have to be highly organized. There is considerable evidence that this is indeed the case. The various cytochrome enzymes have been found to be fixed to the inner membrane (which forms the cristae) of the mitochondria (Fig. 8.6).

The burning of 180 gm of glucose (one **mole**) yields 686,000 calories of energy in the form of heat. One mole of ATP stores about 7300 calories of energy. At this point in our story, one glucose molecule has yielded six molecules of ATP. Two of these represent the net yield from the anaerobic breakdown of glucose. The other four were produced as two *pairs* of electrons (one pair from each PGAL) passed through the cytochrome sys-

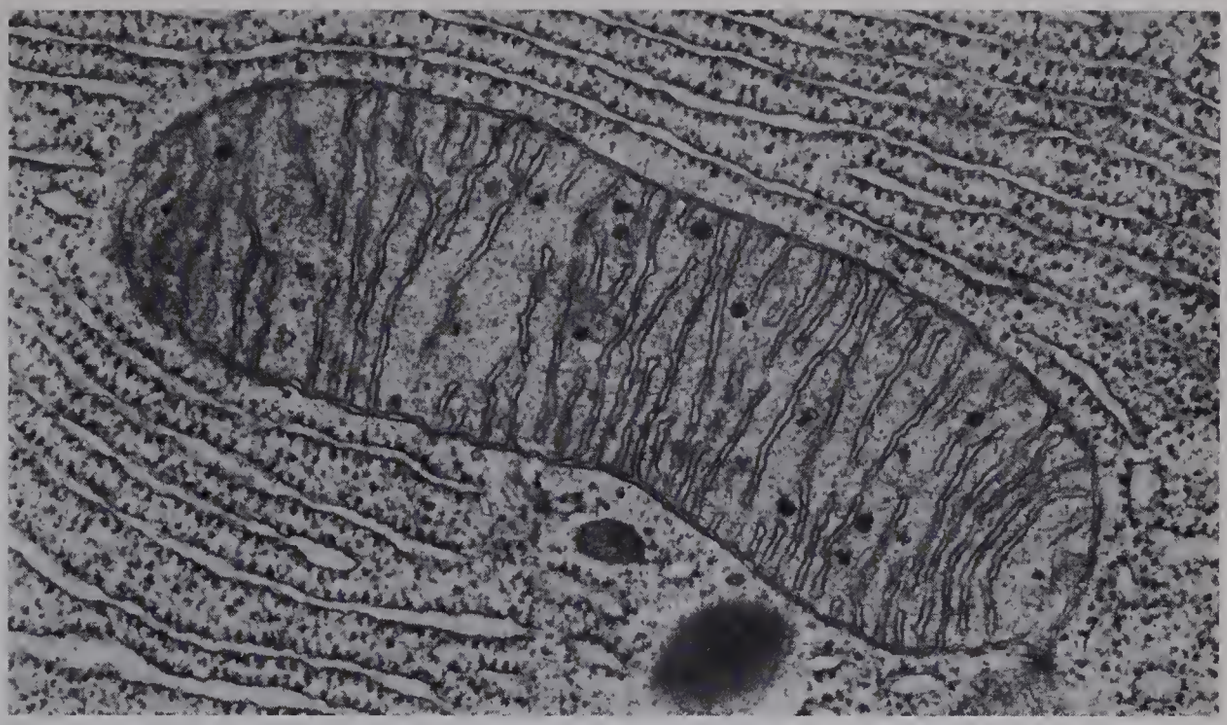


Fig. 8.6 Organization of mitochondria. The way in which the inner membrane projects into the interior to form the cristae shows clearly in this mitochondrion from the pancreas of a bat. (70,000 X, courtesy of Keith R. Porter.)

tem. NAD-linked oxidations that occur *within* the mitochondria yield three ATP molecules for each pair of electrons. However, in the oxidation of PGAL, which occurs in the cytoplasm, the *first* step of ATP production is bypassed and only two ATP's are produced for each pair of electrons. Thus less than 7% (approximately 44,000 calories per mole) of the energy present in glucose has been extracted in the formation of pyruvic acid. The explanation lies in the fact that pyruvic acid itself is a reasonably complex, energy-rich molecule. The oxidation of pyruvic acid is the next part of our story. It takes place within the mitochondria.

Most of the potential energy stored in glucose is still present in pyruvic acid

8.5 THE CITRIC ACID CYCLE

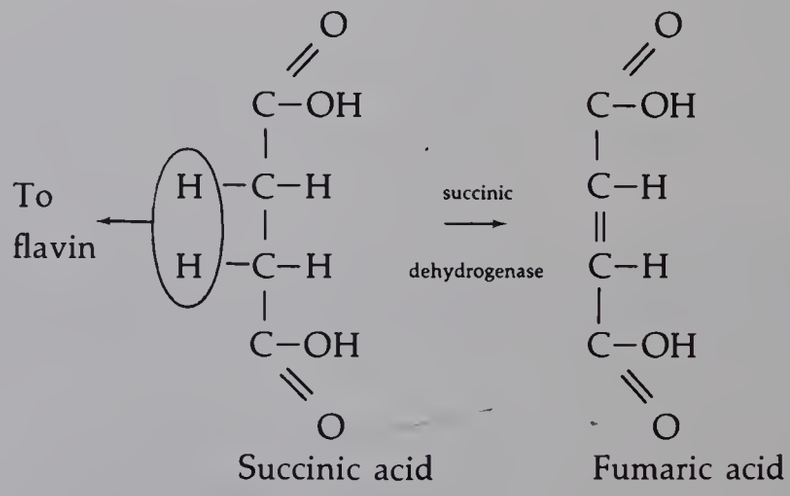
To extract the maximum amount of energy from a molecule of glucose, the glucose must be oxidized completely to CO_2 and H_2O. In converting glucose into two molecules of pyruvic acid, only a partial oxidation has been achieved: four electrons (and their associated H^+ ions) have been removed from the molecule and passed into a mitochondrion and through the cytochrome enzyme system. But the process of cellular respiration does not stop with the formation of pyruvic acid. Instead, the molecule itself enters a mitochondrion and is oxidized still further until only CO_2 and H_2O are left. This oxidation of pyruvic acid goes on in a step-by-step fashion just as does the breakdown of glucose to pyruvic acid. Each step in the process requires the presence of a specific enzyme. These are localized in the membranes of the mitochondria as are the cytochrome enzymes.

The enzymes of the citric acid cycle are confined to the mitochondria

Coenzyme A

The first step in the oxidation of pyruvic acid is the removal of a molecule of CO_2 (Fig. 8.4). The residue is then "activated" by being combined with a coenzyme, called coenzyme A. This complex molecule is called active acetate. In the process of activation, two electrons (with H^+'s) are transferred to NAD and from NAD into the cytochrome system. With the aid of a molecule of H_2O, the active acetate complex is broken, coenzyme A is regenerated, and the acetate portion of the molecule ($C_2H_4O_2$) is united with a molecule of oxaloacetic acid ($C_4H_4O_5$). The new molecule ($C_6H_8O_7$) is **citric acid.** (This is the acid which is found in citrus fruits—lemons, grapefruit, etc. It has provided the name for the sequence of chemical changes we are discussing.) After a series of enzyme-catalyzed molecular changes, including removal of another molecule of CO_2 and the removal of another pair of electrons by NAD, alpha-ketoglutaric acid is formed. Another molecule of CO_2 and two more electrons (with H^+'s) are removed from the alpha-ketoglutaric acid by NAD. A molecule of H_2O enters the reaction at this step. The result of these changes is succinic acid. The molecular rearrangements involved in the conversion of alpha-ketoglutaric acid to succinic acid also result in the production of one molecule of ATP.

Two more electrons (and H^+'s) are removed from succinic acid to form fumaric acid.



The oxidizing agent in this case is flavin. Do you suppose that as many molecules of ATP are produced when flavin donates electrons to the cytochrome system as when $NADH_2$ does? (Figure 8.4 can help you answer this.)

The addition of a molecule of H_2O to fumaric acid produces malic acid. NAD then removes two electrons (and two H^+'s) from malic acid with the formation of oxaloacetic acid. What do you suppose then happens to the oxaloacetic acid that has been formed?

The citric acid cycle has come full circle

In passing through the chemical changes of the citric acid cycle, acetate has been completely oxidized to H_2O and CO_2. When the process is completed, the "carrier" molecule, oxaloacetic acid, is regenerated and can then pick up another molecule of acetate.

8.6 IMPORTANCE OF CELLULAR RESPIRATION

You may well ask what is the significance of oxidizing glucose by such an involved sequence of chemical reactions. The answer should be clear once we see just how much ATP is produced during the respiration of one molecule of glucose (Fig. 8.4). Remember that a single molecule of glucose gives rise to two molecules of PGAL, whose fate we have just examined. At five different times in the breakdown of one PGAL molecule, NAD removes a pair of electrons and passes them through the cytochrome system. When this occurs within the mitochondria (four times), each pair of electrons gives rise to three molecules of ATP. However, the oxidation of PGAL itself, which takes place in the cytoplasm, yields only two molecules of ATP. Hence 14 molecules of ATP are produced from each PGAL molecule by this process, making a total of 28 ATP's for each glucose molecule. In addition one pair of electrons is removed by flavin, but these give rise to only two ATP molecules, since they pass down a shorter length of the chain of enzymes on the mitochondrial membranes. The shortage is made up, however, by the single ATP produced in the transformation of alpha-ketoglutaric acid into succinic acid. Again, as the cycle must "turn" twice for each glucose molecule oxidized, a total of six more ATP molecules are produced by these mechanisms. Adding the net yield of two molecules of ATP which are produced when glucose is broken down anaerobically to pyruvic acid, we end up with a grand total of 36 molecules of ATP produced by the oxidation of a single molecule of glucose.

The respiration of a single molecule of glucose gives rise to 36 molecules of ATP

As mentioned in Section 8.4, a mole of glucose yields 686,000 calories of energy when fully oxidized. In the rapid, uncontrolled oxidation of burning, all this energy is liberated as heat and light. The controlled, step-by-step oxidation of glucose in cellular respiration, in contrast, permits a substantial part of this energy to be conserved in the form of chemical energy. This chemical energy, stored in the high-energy phosphate bonds of ATP, is then used to carry out the many energy-consuming activities of the cell. The efficiency of the process can be calculated.

Calculating the efficiency of cellular respiration

The conversion of a mole of ADP to ATP requires about 7300 calories. The respiration of a mole of glucose gives rise to 36 moles of ATP. Thus about 263,000 calories are conserved, or 38–39% of the energy (686,000 calories) available in the glucose molecules. An efficiency of 39% in the conversion of the chemical energy of glucose into the chemical energy of ATP compares very favorably with the 15–30% efficiency of internal combustion engines in converting chemical energy into mechanical energy.

Not only can we set up a balance sheet to study the energy conversion process, but we can also set up a balance sheet indicating the substances

consumed and the substances produced by respiration. Obviouslv we start with one molecule of glucose. As this is broken down, it gives rise to 12 pairs of electrons (and H^+'s) which pass through the cytochrome enzyme system and unite with 6 molecules of oxygen to form 12 molecules of H_2O. Although only 12 hydrogen atoms and 6 oxygen atoms are present in the original glucose molecule, the deficit is made up by the introduction of 6 (three at each turn of the cycle) water molecules into the citric acid cycle. Six molecules of CO_2 are also produced during these transformations, three from each pyruvic acid molecule entering the cycle. Thus the overall chemical change in cellular respiration can be expressed by the equation:

$$C_6H_{12}O_6 + 6O_2 + 6H_2O \longrightarrow 6CO_2 + 12H_2O$$

See whether you can verify the correctness of this equation by studying Fig. 8.4.

The intermediates in cellular respiration link protein, fat, and carbohydrate metabolism

The step-by-step oxidation of glucose into H_2O and CO_2 has another important function. Several of the *intermediate* compounds formed in the process link glucose metabolism with the metabolism of other foodstuffs. Thus PGAL is a breakdown product of glycerol (a component of fats) as well as an intermediate in the breakdown of glucose. Active acetate is produced by the breakdown of the fatty acid component of fats. It is also produced when certain amino acids have their nitrogen removed in the process of **deamination.** Pyruvic acid, alpha-ketoglutaric acid and oxaloacetic acid also may be formed from amino acid breakdown. These links thus permit the oxidation of excess fats and proteins in the diet. No special mechanism of cellular respiration is needed by those animals that depend largely on ingested fats (e.g. many birds) or proteins (e.g. carnivores) for their energy supply. These links between carbohydrate metabolism and the metabolism of fats and proteins are useful in still another way. Fatty acids, glycerol (and then fats), and some amino acids can be synthesized using these intermediates. As we have seen, a human can become fat as a result of eating excess amounts of carbohydrates. A knowledge of the links between carbohydrate metabolism and fat metabolism shows us how this is possible (see Fig. 8.4).

The steps in the processes of fermentation and cellular respiration are also interesting because they are found in such a wide variety of living things. While some differences have been found in different organisms, most of the enzymes and intermediate compounds in the processes are common to almost all organisms from bacteria to men.

8.7 HOW THESE DISCOVERIES WERE MADE

You may be puzzled as to how biochemists have been able to discover all the intermediate steps in fermentation and cellular respiration. The discovery has required many years, the work of many men, and the use of a variety of ingenious techniques.

One technique in studying intermediary metabolism is to supply a suspected intermediate substance and see (1) whether it is used up and (2)

The biochemist uses a variety of tactics to discover the pathways of intermediary metabolism

what new substance begins to accumulate. Sometimes this technique is applied to the intact organism, but usually it is applied to isolated organs, tissues, or even cell extracts. The discovery by Hans Krebs that oxaloacetic acid ($C_4H_4O_5$) added to isolated pigeon breast muscle is converted into citric acid ($C_6H_8O_7$) enabled him to work out the reactions in the citric acid cycle or, as it is sometimes called, Krebs' cycle.

Another technique is to attempt the isolation of a specific enzyme. Grinding up a piece of tissue, such as liver tissue, releases the cell contents. The biochemist can then attempt to extract and purify a single enzyme. If this is accomplished, he can then determine the single substrate upon which that enzyme acts and the substance produced by its action.

The use of enzyme poisons has also provided information on intermediary metabolism. A poison that interferes with cellular respiration may actually affect only a single enzyme in the process. For example, the poisonous effect of malonic acid (see Section 6.8) is due to its inhibition of succinic dehydrogenase, the enzyme that catalyzes the conversion of succinic acid to fumaric acid in the citric acid cycle. When such a situation occurs, the normal substrate (succinic acid in this case) of the poisoned enzyme accumulates and may be identified. Then if the substance normally *produced* by that enzyme can be discovered, using the techniques mentioned above, this substance can be added to the poisoned system. If respiration begins again, as it does when fumaric acid (Fig. 8.4) is added to tissues poisoned by malonic acid, another link in the chain has been established.

One of the best techniques for studying intermediary metabolism is to introduce into an enzyme system molecules which have been "tagged" with radioactive atoms. The gradual appearance of radioactivity in other chemical substances will indicate the pathway by which the chemical changes have occurred. In the next chapter, we shall see how this technique was used to determine one of the important steps in the process of photosynthesis.

8.8 THE USES OF ENERGY

The energy-consuming and energy-releasing reactions of the cell are linked by ATP

The processes of cellular respiration and fermentation transform chemical energy stored in food molecules into chemical energy stored in the high-energy phosphate bonds of ATP. ATP, in turn, serves as the immediate source of energy for all the energy-requiring activities of the organism. These fall into several categories.

1. Mechanical Work. One of the most important ways in which energy is used by living organisms is to carry on mechanical work. This is especially true of animals. Running, swimming, flying, and climbing are just a few common animal activities in which work is accomplished and energy is consumed. In these cases, the mechanical work is brought about by the contraction of special cells, the muscle cells. The force exerted when muscle cells contract can then be transmitted to organs of locomotion, etc.

ATP supplies the energy for muscular contraction



Fig. 8.7 A culture of luminescent bacteria photographed by their own light. (Courtesy of the Carolina Biological Supply Company.)

The energy needed to accomplish muscular contraction is supplied by ATP.

The beating of cilia and flagella is also powered by ATP and accomplishes mechanical work. Such action may propel the cell through the medium as the flagella of *Chlamydomonas* do (Fig. 5.16). Ciliated stationary cells move the medium past them. The cells lining our air passages, for example, are ciliated. Their cilia sweep inhaled dust particles, etc. back up to the throat.

2. Electrical Work. Energy is also used by living things to accomplish electrical work. The electric eel is a dramatic example of this. Special electric organs in this fish are capable of converting the energy of ATP into a brief current sufficient to stun an adult human. On a less dramatic (but far more important) scale, the energy of ATP is used to create the voltage which exists between the interior and exterior surfaces of most cell membranes. All nervous activity depends upon this kind of electrical activity.

Anything which interferes with ATP production also inhibits active transport

3. Active Transport. A third way in which cells use energy is to counteract the passive forces of diffusion. The active transport of ions or molecules from regions of low concentration to regions of high concentration seems to be closely linked to the production and use of ATP by the cell. Several specific examples of active transport were mentioned in Section 6.5. The periodic filling (and contraction) of the contractile vacuole of the amoeba, for example, requires the energy of ATP.

4. Bioluminescence. Many organisms are able to give off light, and light, of course, is a form of energy. Although the firefly is a common example, the majority of luminescent organisms are found in the oceans. These include certain marine bacteria (Fig. 8.7), protozoans, and many deep-dwelling invertebrates and fishes. Although the mechanism of bioluminescence is incompletely understood, the immediate source of energy for the process is ATP.

5. Heat Production. Energy is also a source of heat for living things. Mammals and birds are especially dependent upon internally-generated heat. They can maintain a fixed body temperature despite fluctuations in the environmental temperature. Generally, the production of heat occurs simply as a by-product of other energy transformations within the cell. As we have already seen, no energy transformation is 100% efficient. For example, when chemical energy is converted into mechanical energy (as in muscular contraction) a substantial amount (70–80%) of the energy is lost in the form of heat. The involuntary muscular contraction which we call shivering exploits this inefficiency to prevent our body temperature from falling below normal. Thus although heat cannot do work for an organism, its production may be vital to the organism in cold surroundings.

Anabolic reactions run on the energy of ATP

6. Anabolism. Anabolism is the synthesis of macromolecules such as proteins, polysaccharides, and nucleic acids from small precursors. Such syntheses cannot proceed without the input of energy. Directly or indirectly, ATP is the energy source for all the anabolic activities of cells.

The products of anabolism

The products of anabolism serve a number of essential functions. Glycogen and fats and even proteins serve as reserve fuels for later catabolism. The synthesis of new nucleic acids enables the organism to store and express additional copies of its library of genetic information. Protein, protein-carbohydrate, and protein-lipid molecules are essential structural components of the organism, both intracellular and extracellular. When synthesis of these materials proceeds at a faster rate than their degradation, the organism grows.

All anabolic transformations are catalyzed by enzymes just as catabolic reactions are. In view of the general reversibility of enzyme-catalyzed reactions, we might expect that the enzymes involved in catabolism could also serve in anabolism by such simple expedients as shifting the relative concentration of substances on each side of the equation. Actually, some

Some anabolic reactions are simply reversals of catabolic reactions

anabolic pathways *do* use some of the same enzymes as related catabolic pathways. The nine enzymatic steps that produce lactic acid from glucose phosphate are readily reversible. One of the functions of the liver is to convert lactic acid back into glucose phosphate using the same enzymes that catalyze the anaerobic breakdown of glucose phosphate (see Section 8.3). However, it seems certain that no metabolic pathway can be completely reversed and a macromolecular product formed without the intervention of some enzymes unique to anabolism. In order for glucose phosphate to be synthesized into *glycogen,* three enzymes are required which are not the same as the enzymes that catalyze conversion of glycogen to glucose phosphate (Fig. 8.4).

All anabolic pathways include some unique steps

Having anabolic and catabolic pathways at least partly separated is good strategy for the cell. Some catabolic reactions are virtually irreversible and an easier route must sometimes be found to get back "up" the metabolic pathway. Furthermore, having separate steps in catabolism and anabolism enables the cell to exert separate control mechanisms over each process. For example, a build-up of glucose phosphate might be expected

Anabolic and catabolic pathways are controlled separately

to lead to increased cellular respiration as a simple consequence of increasing the concentration of a reactant in a reversible reaction (see Section 3.12). But this would be appropriate only if the cell were short of ATP and, in fact, the ATP level does control this catabolic pathway. However, glucose phosphate activates the last enzyme in glycogen synthesis ("precursor activation"—see Section 6.8) and will be drawn off into this anabolic pathway if it is not being used for cellular respiration. It is worth noting that anabolic and catabolic reactions are often physically separated in the cell as well. We have seen the role that mitochondria play in catabolism. Many anabolic reactions require *cytoplasmic* enzymes or enzymes found in the endoplasmic reticulum and elsewhere.

The synthesis of proteins and nucleic acids from their precursors differs in a crucial way from that of polysaccharides and fats. Starch (glycogen) and cellulose are polymers made up of the same monomeric subunit, glucose. A variety of fatty acids may be used in fats, but the structure of the molecules is quite simple. Within limits, these macromolecules are pretty much alike, whatever the organism in which they are found. The situation is quite different for the synthesis of proteins and nucleic acids. These molecules are complex polymers. The absolute sequence in which their monomeric subunits are ordered is crucial to their function. They could not be synthesized by a simple reversal of catabolism because they must be synthesized by a process that assembles their subunits in a specified order. These molecules thus contain *information*—information that is in many cases unique to the organism in which synthesis is taking place. We may catabolize beef proteins or bean proteins, but we synthesize human proteins. Not only that, but some of the proteins we synthesize are unique to us as individuals (unless we have an identical twin!). The mechanisms by which we synthesize precisely ordered sequences of subunits in our nucleic acids and proteins will be described in Chapter 19.

The monomers of proteins and nucleic acids must be assembled in a precisely-ordered sequence

Just as catabolism releases energy, so anabolism requires it. And, of course, it is the energy released by catabolism—in the form of ATP—that powers anabolism. In order for amino acids to be assembled into a protein, they must first be "activated." The energy for this comes from ATP. The monomers of nucleic acids are nucleotides with high-energy phosphate bonds, one of which is, in fact, ATP. The others (i.e. GTP, CTP, UTP for RNA and their *deoxy*-derivatives for DNA) receive their high-energy phosphate bonds from ATP. In order to assemble glucose molecules into starch or cellulose, they must first be activated. ATP provides the energy for this. Synthesis of fats also requires ATP.

ATP is a nucleotide

In our discussion of cellular respiration (Section 8.6) we noted the many intermediates which serve to connect carbohydrate catabolism to the catabolism of fats and proteins (Fig. 8.4). These connecting points between different pathways act as two-way valves. Not only do they provide points of entry for the catabolism (cellular respiration) of fatty acids, glycerol, and amino acids, but for their synthesis as well. Thus the catabolic breakdown of starches can lead (through "active" acetate and PGAL) to the synthesis

Some intermediate metabolites link anabolic and catabolic pathways

of fat—an all-too-common occurrence! Much of the protein we consume is ultimately converted into glucose to provide fuel for the brain and other tissues. Although all our foods are interconvertible to some degree, they are not completely so. In other words, no one food can supply all our anabolic needs. We can indeed synthesize many fats from glucose, but certain fats (unsaturated) cannot be and must be taken in directly in our diet. Although we can synthesize twelve amino acids from carbohydrate precursors, we must have eight others (the "essential" amino acids) included in our diet.

In this chapter and Chapter 7, we have examined how heterotrophic organisms secure and use the complex, energy-rich, organic molecules which we call food. Whether eaten by herbivore or carnivore, the ultimate source of all these organic molecules is the life activities of autotrophic organisms. Only autotrophs can manufacture organic molecules from inorganic raw materials. How they do this is the topic of the next chapter.

EXERCISES AND PROBLEMS

1. Pound for pound, what food yields the most energy when oxidized?
2. In what ways is alcoholic fermentation similar to cellular respiration?
3. In what ways is it different?
4. What parallels can you draw between the process of bread making and the process of brewing? What differences are there?
5. List the substances in the cell that participate in redox reactions.
6. Why is the calorie, a unit of heat energy, also used as a measure of chemical energy?
7. How many moles of oxygen are consumed when two moles of glucose are respired? How many moles of carbon dioxide are produced?
8. What is the ratio of moles of oxygen consumed to moles of CO_2 produced when glucose is respired?
9. What happens to this ratio when fats are used as the fuel in cellular respiration?
10. Why are alcoholic beverages usually brewed in sealed containers?
11. What is the maximum weight of alcohol that yeast cells can produce from 90 gm of glucose?
12. What is the net yield of ATP molecules when glycogen rather than glucose is used to produce the glucose phosphate used in cellular respiration?

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9 PHOTOSYNTHESIS: TRAPPING THE SUN'S ENERGY

Early Experiments

The Pigments

Chloroplasts

The Leaf

Factors Limiting the Rate of Photosynthesis

The Dark Reactions

The Light Reactions

The Chemoautotrophic Bacteria



Fig. 9.1

The preservation of order requires an unceasing input of energy

The life of every living organism depends upon a steady supply of materials and energy. The materials are necessary to provide for the growth and repair of the organism. The energy is necessary to permit the organism to maintain its structure in opposition to the tendency of all things (from molecules—see Section 6.3—to bureau drawers!) to become randomly arranged and disordered. In Chapter 5 we saw how intricately organized is the living cell. Even its constituents, such as the cell membrane, the mitochondria, and the parts of the nucleus, reflect an orderly arrangement of the protein, lipid, and nucleic acid molecules of which they are composed. Cells, in turn, are organized into tissues, the tissues into organs, etc. All these levels of organization depend ultimately upon energy to preserve their pattern. The maintenance of these complex, orderly patterns is one of the main features that distinguish living from nonliving things. With additional energy, additional materials can be organized into these patterns, resulting in growth.

Energy is also necessary for living things to cope with changes in their environment. The activity of nerves and the contraction of muscles permit you to respond to changes in your environment. These activities require energy. Do you see any connection between using energy for these purposes and using energy to preserve the complex organization of matter which we discussed in the last paragraph?

Heterotrophic organisms supply their needs for both matter and energy by taking in complex, energy-rich, organic molecules from their environment. In the last two chapters we examined some of the mechanisms by which these are made available to the cells. We also examined the way in which these molecules serve not only as a source of materials for the repair and growth of the cell but also as a source of energy for it.

Autotrophic organisms manufacture organic molecules from inorganic ones

Where do these complex, energy-rich, organic molecules come from? They are manufactured by green plants and the various kinds of algae. These organisms are capable of synthesizing organic molecules from simple, inorganic materials in the environment such as CO_2 and H_2O. This type of nutrition is referred to as **autotrophic.** Organisms that are capable of autotrophic nutrition not only supply all their own needs for materials and energy but, directly or indirectly, the needs of all heterotrophic organisms.

Figure 9.1 reflects a crucial biological truth: heterotrophic organisms depend for their existence upon autotrophic organisms. We may dine on beefsteak, but the steer dined on grass.

The kind of autotrophic nutrition upon which almost all heterotrophic organisms (including ourselves) depend is photosynthesis. In photosynthesis, light energy is harnessed for the synthesis of organic compounds from inorganic ones.

PHOTOSYNTHESIS

9.1 EARLY EXPERIMENTS



Fig. 9.2 Van Helmont's experiment. Over a five-year period the willow gained more than 164 lb, while the weight of the soil was practically unchanged.

Our present knowledge of photosynthesis is the outcome of experiments performed and theories created during the past 300 years. Perhaps the first experiment designed to explore the nature of photosynthesis was that reported by the Dutch physician van Helmont, in 1648. Some years earlier, van Helmont had placed in a large pot exactly 200 pounds of soil that had been thoroughly dried in an oven. Then he moistened the soil with rain water and planted a five-pound willow shoot in it. He then placed the pot in the ground and covered its rim with a perforated iron plate. The perforations permitted water and air to reach the soil but lessened the chance that dirt would be blown into the pot from the outside (Fig. 9.2). For five years, van Helmont kept his plant watered with rain water or distilled water. At the end of that time, he carefully removed the young tree and found that it had gained 164 pounds, 3 ounces. (This figure did not include the weight of the leaves that had been shed during the previous four autumns.)

He then redried the soil and found that it weighed only 2 ounces less than the original 200 pounds. Faced with these experimental facts, van Helmont theorized that the increase in the weight of the willow arose from the water alone. He did not consider the possibility that gases in the air might also be involved.

The first evidence that gases participate in photosynthesis was reported by Joseph Priestley in 1772. He knew that if a burning candle is placed in a sealed chamber, the candle soon goes out. If a mouse is then placed in the chamber, it quickly suffocates, because the process of combustion has used up the oxygen in the air—the same gas upon which

animal respiration depends. However, Priestly discovered that if a plant is placed in an atmosphere devoid of oxygen, it very soon replenishes the oxygen and a mouse can survive in the resulting mixture. Priestly thought that it was simply the growth of the plant that accounted for this. It was the Dutch physician Ingen-Housz who discovered in 1778 that the effect observed by Priestly occurred only when the plant was illuminated. A plant kept in a sealed chamber in the dark consumes oxygen just as a mouse (or candle) does.

Senebier showed that plants take up CO_2

The growth of plants is accompanied by an increase in their carbon content. A Swiss minister, Jean Senebier, discovered that the source of this carbon is carbon dioxide and that the release of oxygen during photosynthesis accompanies the uptake of carbon dioxide. Senebier's fellow countryman, de Saussure, placed these discoveries on a more quantitative foundation by carefully measuring the amounts of CO_2 consumed and O_2 liberated during photosynthesis. He also showed that plants growing in the presence of carbon dioxide increased their carbon content, while plants growing in an atmosphere devoid of CO_2 actually lost small amounts of carbon. (Seedlings may get larger under these conditions, but de Saussure showed that this resulted from their uptake of water. Without photosynthesis, the growth of the seedling could occur only at the expense of carbon compounds present as food stores in the seed.)

9.2 THE PIGMENTS

Ingen-Housz also showed by experiments that only the green portions of plants liberate oxygen. He found that nongreen portions, such as woody stems, roots, flowers, and fruits, actually consume oxygen in the process of respiration. We now know that this is because photosynthesis can go on only in the presence of the green pigment **chlorophyll.** Chlorophyll is a magnesium-containing compound which shows a distinct chemical relationship to the prosthetic group of the cytochrome enzymes (Fig. 8.5). Its chief distinguishing features are the presence of a magnesium (instead of iron) atom and a side chain of carbon atoms called the phytol group. Actually, several kinds of chlorophyll molecules are known. Chlorophyll *a* is found in all green plants and all algae. Chlorophyll *b* is found in plants and green algae along with chlorophyll *a*. Still other chlorophylls are found in other algae and in the photosynthetic bacteria.

Photosynthesis depends on the presence of one or more kinds of chlorophyll

Many algae contain other pigments in addition to chlorophylls. These impart the special colors we find in the blue-green algae, the red algae, and the brown algae. They are accessory to the process of photosynthesis.

Chlorophyll is a green pigment because it does not absorb green light. White light (such as sunlight) contains light of all the colors of the visible spectrum from red to violet, but all these colors are not absorbed equally by the pigment. It is possible to determine how effectively each color is absorbed by (1) illuminating a solution of chlorophyll with light of a single color and (2) measuring with a sensitive light meter the amount of light that passes through the solution. By repeating this process with colored

Plotting an absorption spectrum

lights of the entire visible spectrum, it is possible to draw an **absorption spectrum** (Fig. 9.3). Note that chlorophyll absorbs light most strongly in the red and violet portions of the spectrum. Green light is very poorly absorbed. Hence when white light shines upon chlorophyll-containing structures, such as leaves, green rays are transmitted and reflected, with the result that the structures appear green.

Carotenoids absorb wavelengths of light that the chlorophylls absorb poorly

Other pigments are found associated with the chlorophylls in green plant cells. Chief among these are the carotenoids. While carotenoids of many colors are known, in plant cells they range in color from yellow to red. Although their presence in green leaves is usually masked by the greater abundance of the chlorophylls, they often become the dominant pigment in flowers and fruits. The red of the ripe tomato and the orange of the carrot are produced by carotenoids. Even in leaves, the carotenoids may become visible. The brilliant yellows of autumn foliage are produced by the carotenoids which become visible as the supply of chlorophyll in the leaf dwindles.

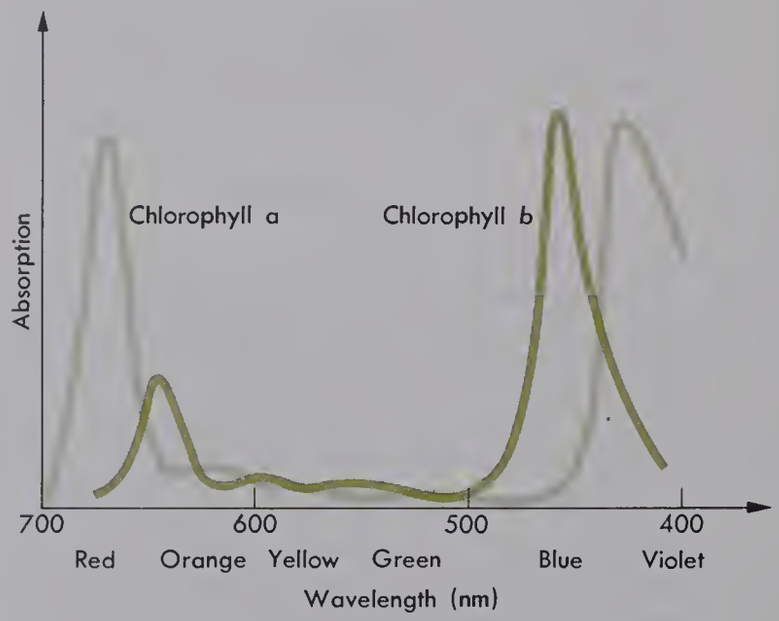


Fig. 9.3 Absorption spectra of chlorophyll *a* and *b*.

In most green plant cells, the carotenoid content is less than one-quarter that of the chlorophylls. This does not necessarily mean that the carotenoids are not important in photosynthesis. However, the relative importance of the two kinds of pigments can be determined by finding out which colors of light are most effective in causing photosynthesis. In 1881, the German plant physiologist T. W. Engelmann performed a series of experiments to discover this. He placed a filamentous green alga under the microscope and illuminated the strand with a tiny spectrum. In the medium surrounding the strands were motile, aerobic bacteria. After a few minutes of illumination, the bacteria were found to be congregated and most active around the portions of the filament illuminated by red and blue light (Fig. 9.4). Assuming that the bacteria were congregating in the regions where oxygen was being evolved, Engelmann concluded that the red rays and the blue rays are the most effective colors for photosynthesis. By

Engelmann used aerobic bacteria to determine the action spectrum of photosynthesis

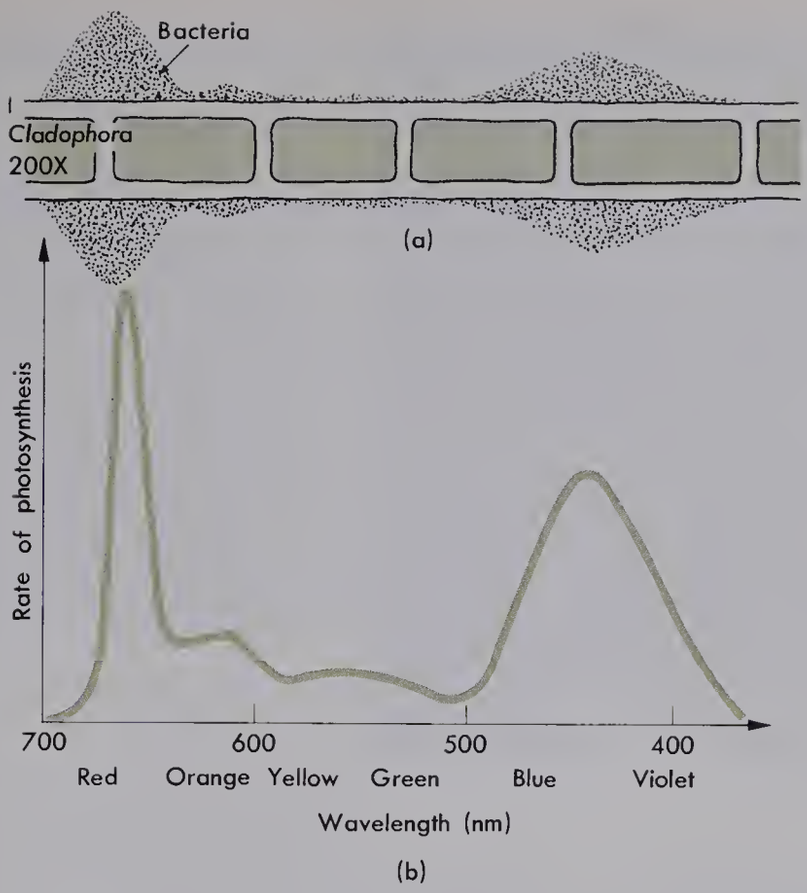


Fig. 9.4 (a) Engelmann's experiment and (b) action spectrum of photosynthesis.

plotting the effectiveness of each color of light in stimulating photosynthesis, one can draw an **action spectrum** (see Fig. 9.4). The similarity of the action spectrum of photosynthesis and the absorption spectrum of chlorophyll suggests that the chlorophylls are the most important pigments in the process. The spectra are not identical, however, and this suggests that the carotenoids play a role in photosynthesis. They appear to help fill in the absorption gaps so that a larger part of the sun's spectrum can be used. The energy absorbed by the carotenoids is passed to chlorophyll *a*, where it is used in photosynthesis. Chlorophyll *b* probably serves a similar function.

9.3 CHLOROPLASTS

Chloroplasts contain all the pigments and enzymes needed to accomplish photosynthesis

The chlorophylls (along with some carotenoids) are contained within cell structures called chloroplasts. In the cells of plants, these are disk-shaped. In some of the green algae they may occur in a variety of shapes. For example, the chloroplast in *Chlamydomonas* is cup-shaped (Fig. 5.16). There are no chloroplasts in the blue-green algae. In these organisms, the pigments are confined to membranes arranged concentrically within the cell (look back at Fig. 5.14).

In addition to the photosynthetic pigments, chloroplasts contain substantial quantities of lipids and proteins. The latter include many enzymes, among them cytochrome enzymes similar to those found in the mitochondria.



Fig. 9.5 Seedlings of the common garden bean grown in light (left) and in darkness (right). The pale color of the dark-grown plant is caused by its lack of chlorophyll. When the food reserves of its seed are used up, the seedling will die. Each seedling shows three nodes, but the internodes are greatly elongated in the dark-grown seedling, a condition known as etiolation.

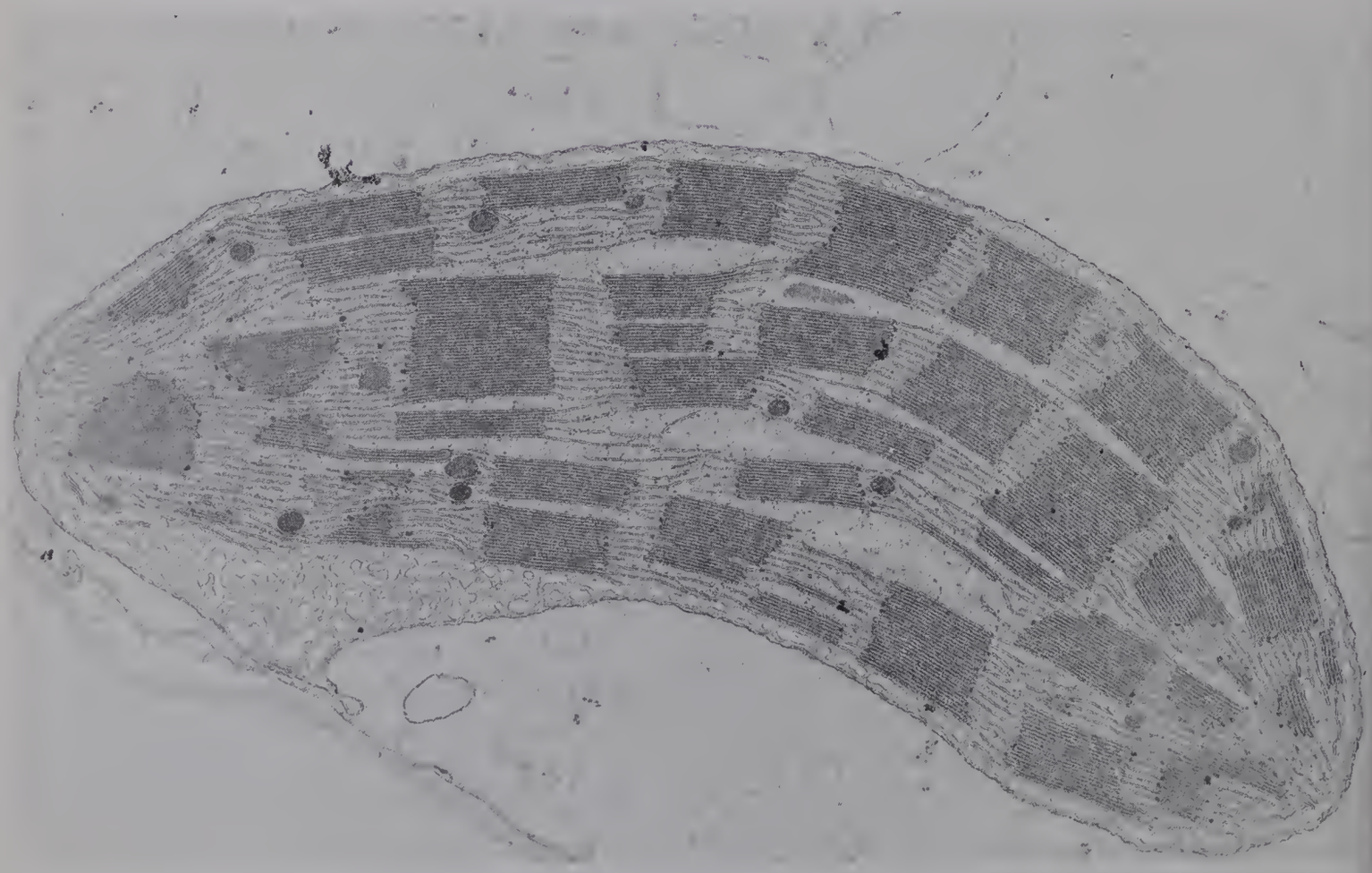


Fig. 9.6 Chloroplast (50,000 X) from a corn leaf cell. The structures that look like stacks of coins are the chlorophyll-containing grana. (Courtesy of Dr. L. K. Shumway, Genetics and Botany, Washington State University.)

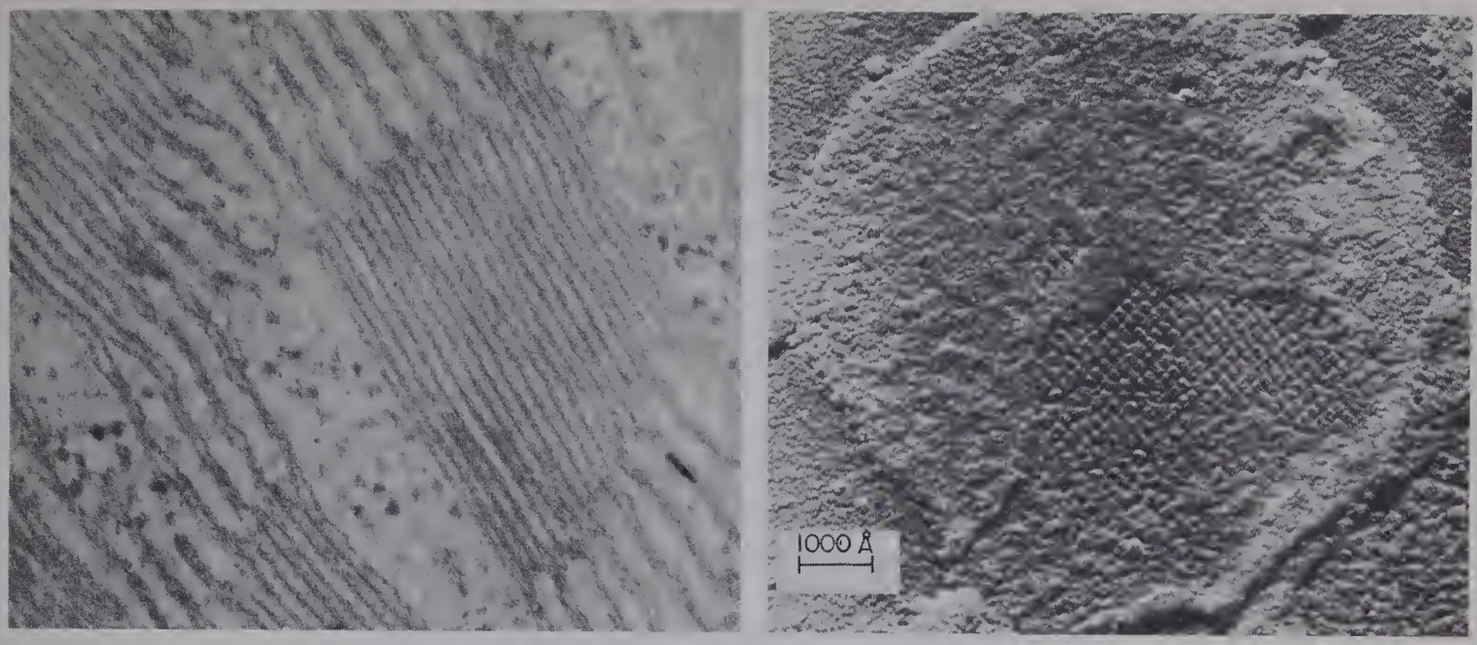


Fig. 9.7 Organization of the chloroplast. Left: under high magnification (60,000 X), the stacks of thylakoids that form the grana can be seen clearly. (Courtesy of Dr. A. E. Vatter.) Right: when viewed on its surface, the thylakoid membrane can be seen to be composed of subunits, the quantasomes. (Courtesy of Dr. R. B. Park from R. B. Park and J. Biggins, *Science,* **144,** 1009–1011, May 22, 1964.)

Chloroplasts arise from tiny, colorless structures in the cells called proplastids. Light is necessary for the development of proplastids into chloroplasts. This explains the pale color of seedlings grown in the dark (Fig. 9.5). Proplastids are capable of duplicating themselves. In fact, this is the only way in which additional proplastids are formed. The nuclei of green plant cells cannot manufacture them. Thus whenever green plant cells undergo mitosis, it is important that both daughter cells receive proplastids in the cytoplasm as well as the normal chromosome content of the nucleus. It is worth noting that these self-duplicating structures also contain DNA and their own protein-synthesizing machinery.

Chloroplasts contain DNA

The disk-shaped chloroplasts of higher plants average about 7 μm in diameter. Under the light microscope, these tiny bodies usually appear to be perfectly uniform in structure. With the aid of the electron microscope, however, it has been found that the chloroplast itself is a highly organized structure (Fig. 9.6). The chlorophylls (and carotenoids) are confined to an internal system of membranes. The membranes occur in pairs connected at their margins, forming an enclosed space. This structure is called a thylakoid (Fig. 9.7). Thylakoids are arranged in stacks, called **grana,** rather like piles of coins. Surrounding the grana is a colorless matrix, the **stroma.** Some of the thylakoids have extensions which provide interconnections between the grana (Fig. 9.7). The membranes of the thylakoids are modified unit membranes. Their components—pigments and enzymes—appear to be organized in orderly arrays of particles, called quantasomes (Fig. 9.7). When isolated from the cell, the chloroplast can be shown to be capable of carrying out the entire process of photosynthesis.

Ultrastructure of chloroplasts

9.4 THE LEAF

In higher plants, chloroplasts are generally confined to parenchyma cells of young stems, immature fruits, and leaves. It is the leaves which are the real photosynthetic factories of the plant. A cross section through the blade of a typical leaf reveals several distinct tissue layers (Fig. 9.8). The top surface of the leaf is covered by a single layer of cells which makes up the **upper epidermis.** These cells contain few or no chloroplasts. They are therefore quite transparent and permit most of the light which strikes them to pass through to the underlying cells. They also secrete a waxy, transparent substance called cutin. This material makes up the cuticle. It serves as a moisture barrier on the upper surface of the leaf, thus reducing water loss from the leaf.

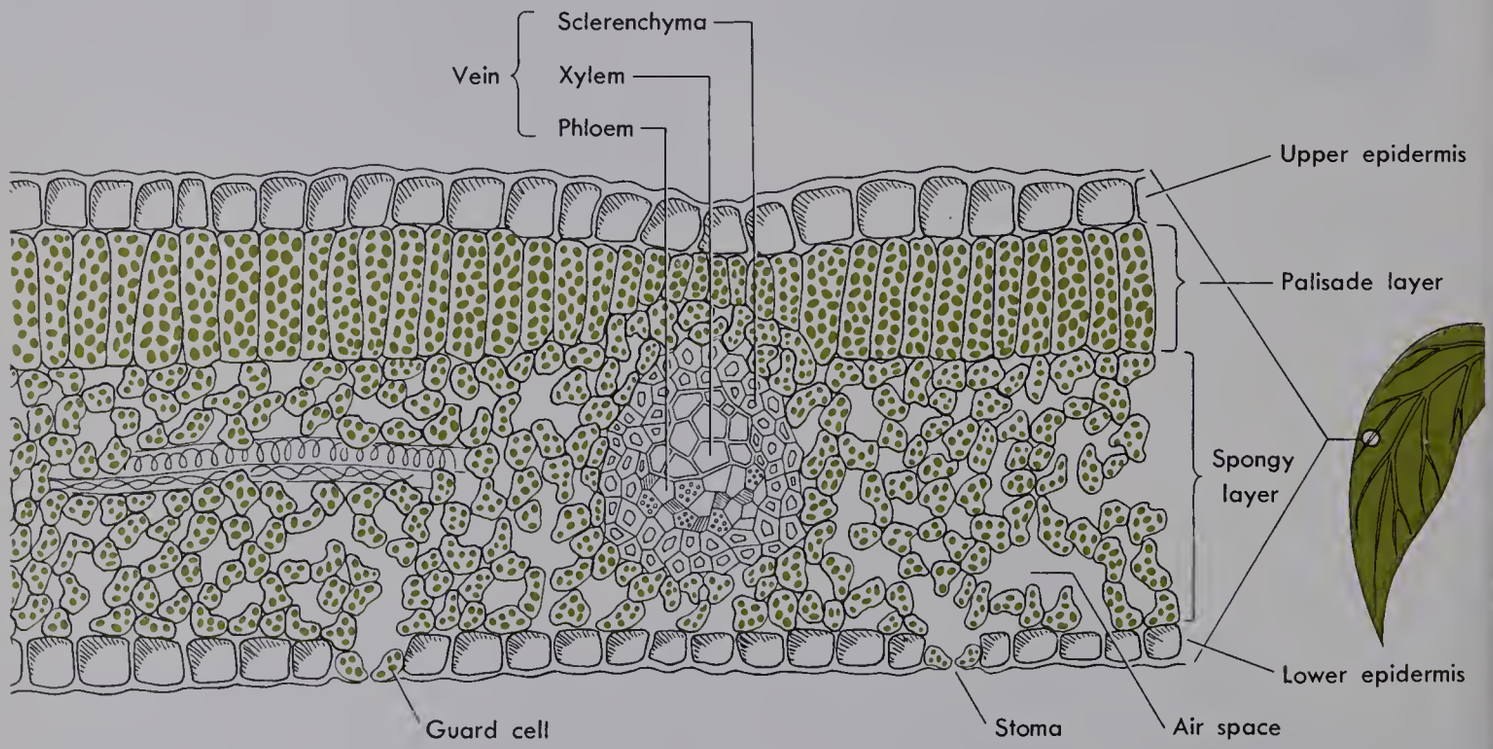


Fig. 9.8 Structure of a typical leaf as seen in cross section. The cells of the palisade layer carry on most of the photosynthesis in the leaf. In some leaves, especially those exposed to bright sun, there may be two or three layers of palisade cells.

Leaves are the food manufacturing organs of the plant

Beneath the cells of the upper epidermis are arranged one or more rows of cells which make up the **palisade layer.** These cells are cylindrical in shape and oriented so that their long axis is perpendicular to the plane of the leaf. Each one is filled with chloroplasts (50 or more) and, as you might guess, these cells carry on most of the photosynthesis in the leaf. The form of the cells is well suited to this function. They are arranged so that a large number of cells are exposed to the sun's rays. Furthermore, their length increases the chance that the light entering the cell will be absorbed by a chloroplast.

Beneath the palisade layer is the **spongy layer** of the leaf. The cells of the spongy layer are irregular in shape and loosely packed. Although they contain a few chloroplasts, their main function seems to be the temporary storage of the food molecules produced by the cells of the palisade layer. They also aid in the exchange of gases between the leaf and the environment. During daylight hours, these cells give off O_2 and water vapor to the air spaces which surround them. They also pick up CO_2 from the air in the air spaces. The air spaces are interconnected with one another and eventually open to the outside of the leaves through special pores called **stomata.**

Gases enter and leave the leaf through stomata

In most plants, the stomata are located chiefly in the **lower epidermis** of the leaf. Ingen-Housz himself first demonstrated that photosynthesizing leaves give off O_2 more rapidly from their lower surface than from their upper surface. The presence of as many as 100,000 stomata per square centimeter in the lower epidermis of some oak leaves and none in the upper epidermis easily accounts for this observation.

Most of the cells of the lower epidermis resemble those of the upper epidermis. Around each stoma, however, are located two sausage-shaped cells called **guard cells.** The guard cells differ from the other cells of the lower epidermis not only in their shape but also in their having large numbers of chloroplasts. The guard cells regulate the opening and closing of the stomata. Thus they exert a considerable control over the exchange of gases between the leaf and the surrounding atmosphere. The mechanism of their action will be considered in Chapter 12.

The leaf also has veins. The veins contain conducting vessels (xylem and phloem) which transport materials to and from the cells of the leaf and the rest of the plant. A detailed analysis of the structure and function of these conducting vessels will be presented in Chapter 14. The conducting vessels of the veins are often surrounded by sclerenchyma cells. These thick-walled cells not only provide mechanical support for the conducting tubes which they surround, but also provide a substantial amount of rigidity to the whole blade of the leaf.

Veins provide a supporting framework for the leaf

9.5 FACTORS LIMITING THE RATE OF PHOTOSYNTHESIS

With the discovery that the first product of photosynthesis to accumulate in any substantial amount is glucose, it became possible to establish an equation for the process:

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2.$$

This equation shows the relationship between the substances used in and produced by photosynthesis. It does not, however, tell us anything about the intermediate steps in the process. That there are intermediate steps was first pointed out by the British plant physiologist F. F. Blackman. You, too, can easily repeat his experiment, using the apparatus shown in Fig. 9.9. The green water plant *Anacharis densa* (available wherever aquarium supplies are sold) is the test organism. When a sprig is placed upside down in a

Blackman demonstrated that photosynthesis occurs in at least two steps: one that requires light and one that does not

dilute solution of $NaHCO_3$ (which serves as a source of CO_2) and illuminated with a flood lamp, oxygen bubbles are soon given off from the cut portion of the stem. You then count the number of bubbles given off in a definite interval of time at each of several light intensities. Plotting this information should produce a graph similar to that in Fig. 9.10. That the rate of photosynthesis does not continue to increase indefinitely with increased illumination led Blackman to the conclusion that at least two distinct processes are involved: one, a reaction that requires light and the other, a reaction that does not. This latter is called a "dark" reaction although it *can* go on in the light. Blackman theorized that at moderate light intensities, the "light" reaction limits or "paces" the entire process. In other words, at these intensities the dark reaction is capable of handling all the intermediate substances produced by the light reaction. With increasing light intensities, however, a point is eventually reached when the dark reaction is working at maximum capacity. Any further illumination is ineffective and the process reaches a steady rate.

The light reactions are controlled by light intensity

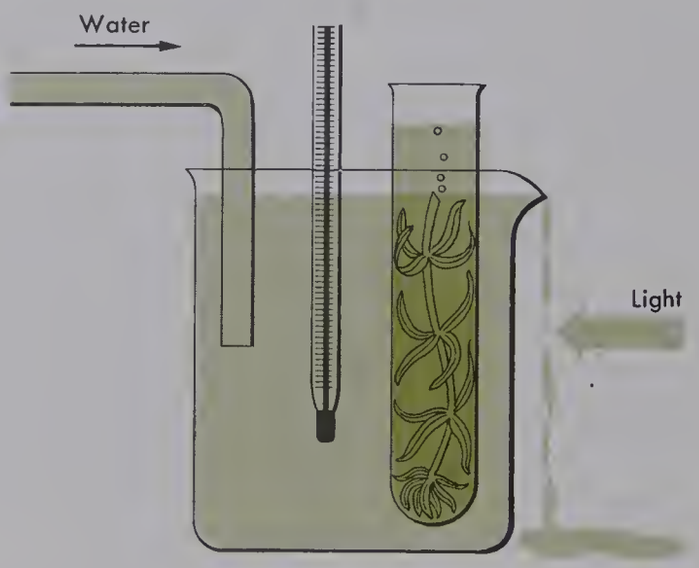


Fig. 9.9 Apparatus for determining the rate of photosynthesis in *Anacharis*. Measurements are made by counting the rate at which bubbles of oxygen are given off at the stem.

The dark reactions are sensitive to temperature and concentration of CO_2

This theory is strengthened by repeating the experiment at a somewhat higher temperature. As we have mentioned, most chemical reactions proceed more rapidly at higher temperatures (up to a point). At 35°C, the rate of photosynthesis does not level off until greater light intensities are present. This suggests that the dark reaction is now working more rapidly. The fact that at low light intensities the rate of photosynthesis at 35°C is no greater than at 20°C also supports the idea that it is a light reaction which is limiting the process in this range. All the light reactions known to chemists depend, not on the temperature, but simply on the intensity of illumination.

The increased rate of photosynthesis with increased temperature does not occur if the supply of CO_2 (in the form of HCO_3^-) is limited. As illustrated in Fig. 9.10, the overall rate of photosynthesis reaches a steady value at lower light intensities if the amount of CO_2 available is limited.

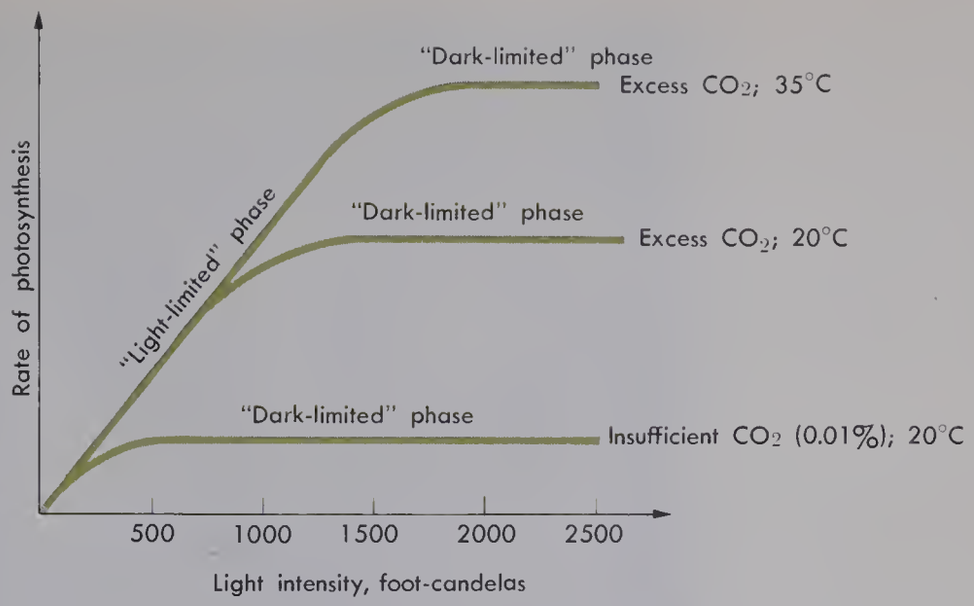


Fig. 9.10 Rate of photosynthesis as a function of light intensity, CO_2 concentration, and temperature. At low light intensities, light is the limiting factor. At higher light intensities, temperature and CO_2 concentration are the limiting factors.

Thus CO_2 concentration must be added as a third factor regulating the rate at which photosynthesis occurs. As a practical matter, however, the concentration of CO_2 available to terrestrial plants is simply that found in the atmosphere: 0.03%

9.6 THE DARK REACTIONS

How Calvin used $^{14}CO_2$ to identify the dark reactions

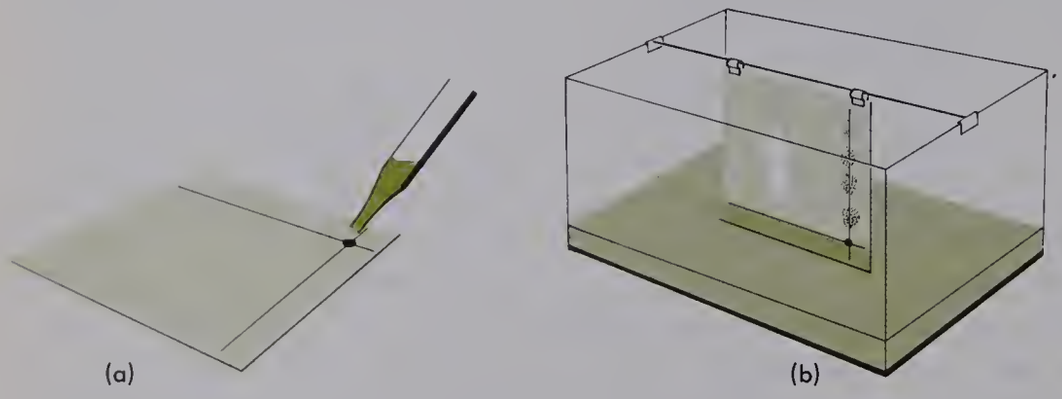
As you might expect, the dark reaction in photosynthesis is actually a series of reactions. These reactions involve the uptake of CO_2 by the plant and the reduction of CO_2 by hydrogen atoms. Dr. Calvin and his associates at the University of California have devoted years to working out the step-by-step sequence of chemical reactions involved. Their basic experimental procedure has been to expose suspensions of unicellular green algae to light and to radioactive carbon dioxide (Fig. 9.11). The use of radioactive carbon (^{14}C) in the carbon dioxide "tags" the atom and allows its chemical transformations to be studied. After various intervals of illumination, the algal suspension is inactivated and the contents of the cells extracted.

Paper chromatography

These are then separated by a process called paper chromatography. A drop of the cell extract is placed along one edge of a square of absorbent paper. The paper is then dipped into a solvent. The solvent migrates up the sheet because of capillary attraction (Fig. 9.12). As it does so, the chemical substances in the drop of cell extract are carried along at different rates. Generally, each compound migrates at a unique rate in a given solvent. When the process is completed, the various substances will be separated at distinct places on the sheet of paper, thus forming what is called a *chromatogram.* The identity of each substance may be determined simply by comparing its position with the positions occupied by known substances under the same conditions. Or, the particular portion of the paper can be cut from the sheet and delicate analytical tests run on the tiny amount of substance present.



Fig. 9.11 Apparatus used to follow the fate of $^{14}CO_2$ in the dark reactions. The algal suspension is placed in the "lollypop," supplied with $^{14}CO_2$, and illuminated. The dark reactions are halted by draining the contents of the lollypop into a flask of hot alcohol. (Courtesy of Dr. James A. Bassham.)



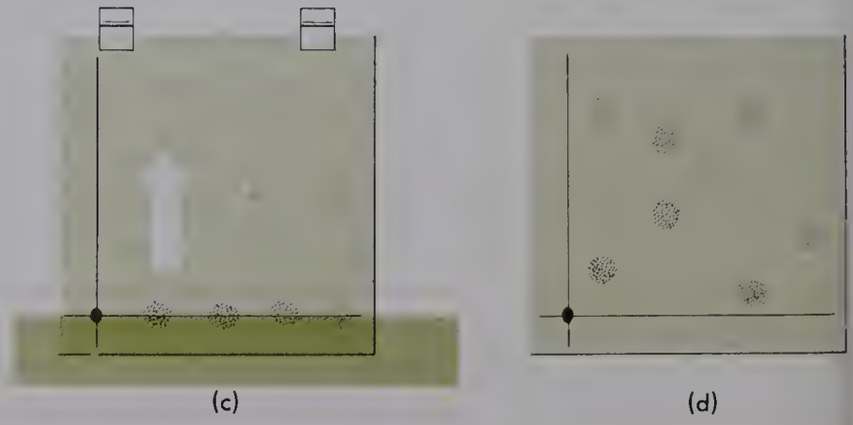


Fig. 9.12 Separation of a mixture by two-dimensional paper chromatography. (a) and (b) Placed in a suitable solvent, the substances in the drop of material at the lower right-hand corner of the paper will be partially separated as they migrate upward. (c) and (d) Further separation can be achieved by turning the paper 90° and using a different solvent.

Radioautography

To determine which, if any, of the substances separated on the chromatogram are radioactive, a sheet of x-ray film is placed next to the chromatogram. If dark spots appear on the film (because of the radiation emitted by the ^{14}C atoms), their position can be correlated with the positions of the chemicals on the chromatogram. Using this technique of *radioautography,* Calvin found that ^{14}C turned up in glucose molecules within 30 seconds after the start of photosynthesis. When he permitted photosynthesis to proceed for only five seconds, however, he discovered radioactivity in many other, smaller, intermediate molecules (Fig. 9.13). Gradually the pathway of carbon fixation was established.

One of the key substances in this process is the five-carbon sugar ribulose phosphate. When it is "activated" by ATP, the resulting compound, ribulose diphosphate, is capable of combining with CO_2. The resulting six-carbon sugar molecule then breaks down to form two molecules of phosphoglyceric acid (PGA) (Fig. 9.14). Then, in what is fundamentally a reversal of the reaction in cellular respiration (see Section 8.3), the PGA molecule is reduced by $NADH_2$ to form phosphoglyceraldehyde (PGAL). (The energy of ATP is also needed to "drive" this reduction.) Starting with three molecules of ribulose phosphate, six molecules of PGAL are formed. Of these six, five are used to re-form the three starting molecules of ribulose phosphate. The remaining PGAL molecule, whose three carbon atoms represent the net gain from one turn of the cycle, stands right at the crossroads of cell metabolism. It can enter the mitochondria and continue "down" the pathway of cellular respiration. This will lead to the production of energy for the cell and/or the production of intermediates that can be used immediately for amino acid and lipid synthesis. On the other hand, an excess of PGAL will tend to reverse the reactions of cellular respiration. The PGAL then travels "up" the pathway to form fructose and then its isomer, glucose. Glucose and fructose can be combined to form the common table sugar, sucrose. This can then be transported to other regions of the plant. Excess glucose can be stored as the polysaccharide starch.

The interconversion of PGA and PGAL occupies a key position in both photosynthesis and cellular respiration

When the techniques used by Calvin have been applied to certain plants (e.g. sugar cane, corn), the first products seen are 4-carbon compounds such as oxaloacetic acid and malic acid rather than the 3-carbon PGA. Soon, however, radioactive carbon begins to appear in PGA and the pathway we have been discussing comes into play. There is some evidence that these plants fix carbon by the 4-carbon pathway in certain cells while retaining the PGA pathway in other cells.

Some plants incorporate CO_2 in 4-carbon compounds

Hardly had the dark reactions in plant photosynthesis been worked out when it was discovered that these dark reactions are not unique to plants at all. The process of reducing carbon dioxide to form glucose is essentially the reverse of the anaerobic stage of cellular respiration. It has been found to go on in a variety of animal cells, including those of the human liver. However, just as the anaerobic breakdown of glucose produces energy (ATP and $NADH_2$), so its reversal *requires* ATP and $NADH_2$. (As you can see from Fig. 9.14, nine molecules of ATP and six of $NADH_2$ are needed for a net gain of one PGAL molecule.) Animal cells can

The dark reactions also occur in heterotrophs

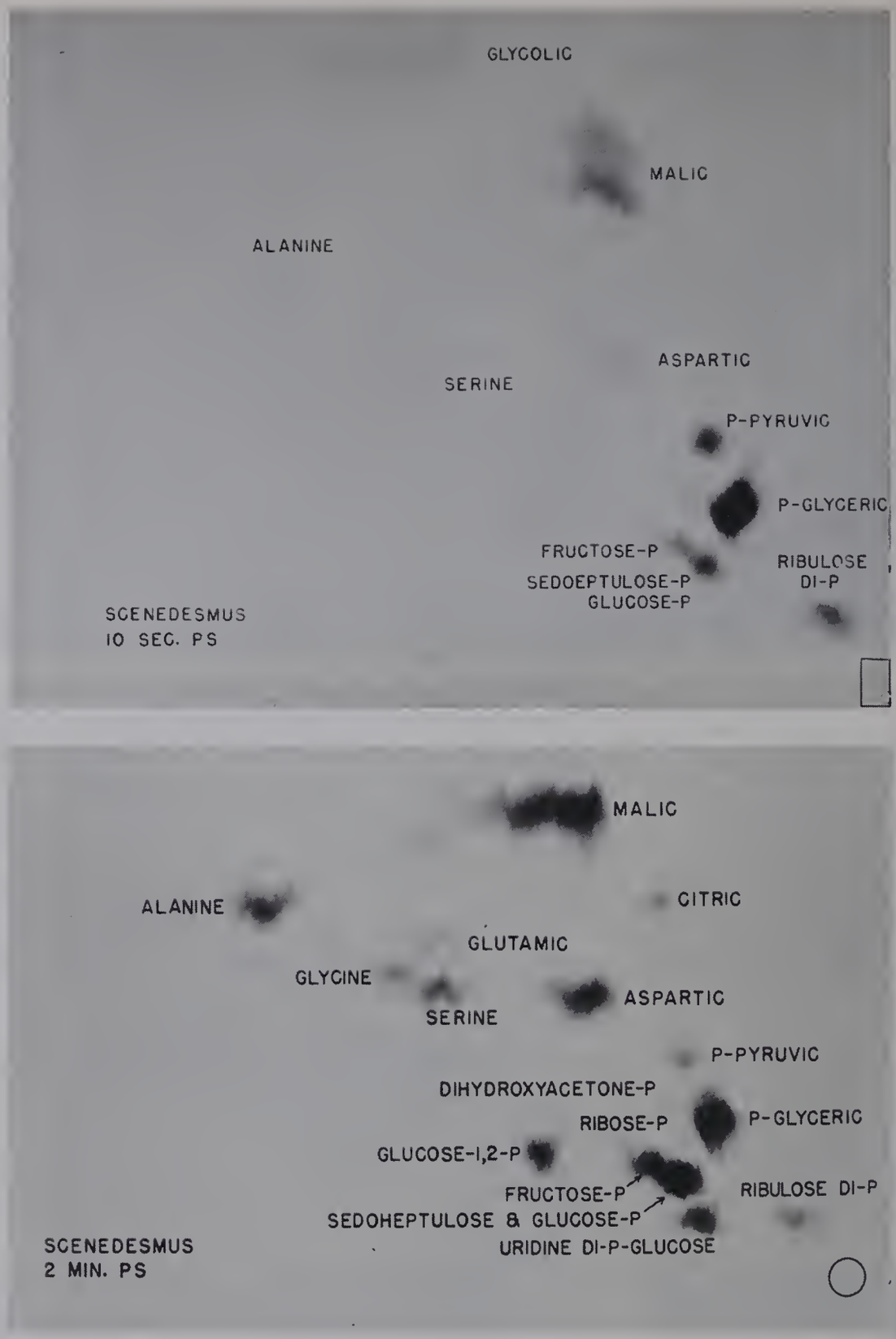


Fig. 9.13 Radioautographs showing the radioactive substances produced after 10 sec (top) and 2 min (bottom) of photosynthesis using radioactive carbon dioxide. At 10 sec, most of the radioactivity is found in PGA ("p-glyceric"). At 2 min, radioactive 6-carbon sugars (glucose and fructose) have been synthesized as well as a number of amino acids. The small rectangle and circle (lower right-hand corners) mark the spots where the cell extract was applied. (Courtesy of J. A. Bassham.)

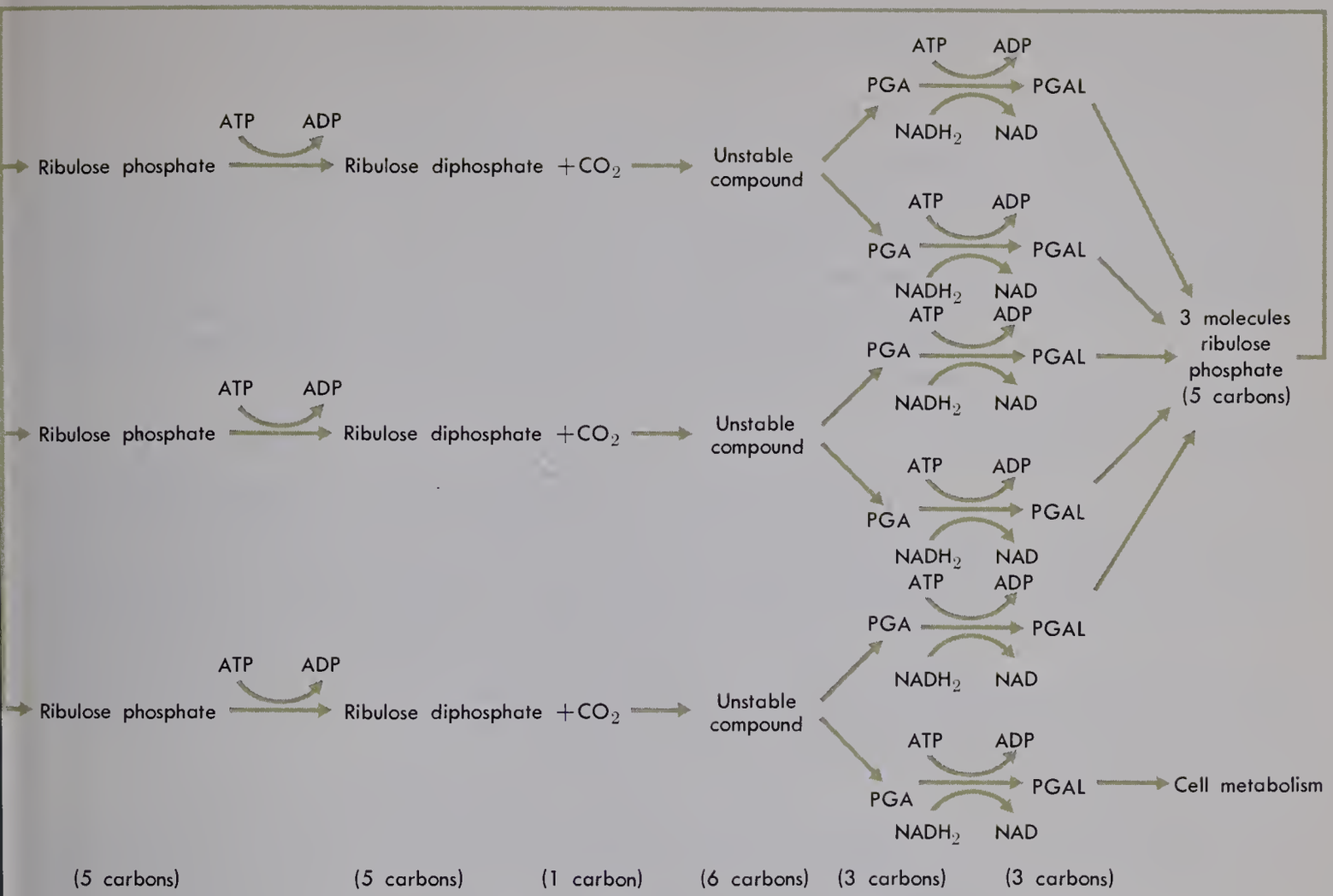


Fig. 9.14 Pathway of carbon fixation in photosynthesis: the "dark" reactions.

thus never hope to accumulate glucose by this method. Remember that they must respire glucose to get their ATP and $NADH_2$ and that the process is only about 40% efficient.

The discovery that the dark reactions of photosynthesis are not unique to green plants does, however, focus our attention on the light reaction. The secret of autotrophism must lie here.

9.7 THE LIGHT REACTIONS

It was the American microbiologist Van Niel who first glimpsed the role that light plays in photosynthesis. He arrived at his theory through studying photosynthesis in the purple sulfur bacteria. These microorganisms produce glucose from CO_2 as do the green plants, and they need light to accomplish the synthesis. Water, however, is not used as a starting material. Instead, these bacteria use a gas (which is poisonous to us), hydrogen sulfide (H_2S). Furthermore, no oxygen is liberated during this photosynthesis but rather the element sulfur. Van Niel reasoned that the action of light caused a decomposition of H_2S into hydrogen atoms and sulfur. Then,

The purple sulfur bacteria use H_2S as a source of electrons for photosynthesis

in a series of dark reactions, the hydrogen atoms were used to reduce CO_2 to carbohydrate:

$$CO_2 + 2H_2S \longrightarrow (CH_2O) + H_2O + 2S.$$

(The parentheses around CH_2O signify that no specific molecule is being indicated but, instead, the ratio of atoms in some more complex molecule, e.g. glucose, $C_6H_{12}O_6$.)

Van Niel's theory

In these reactions Van Niel envisioned a parallel to the process of photosynthesis in green plants. He reasoned that in green plants the energy of light causes water to break up into H_2 and O. The hydrogen atoms are then used to reduce CO_2 in a series of dark reactions:

$$CO_2 + 2H_2O \longrightarrow (CH_2O) + H_2O + O_2.$$

If this theory is correct, it follows that all of the oxygen produced in photosynthesis is derived from water just as all the sulfur produced in bacterial photosynthesis is derived from the H_2S. This conclusion directly contradicts an earlier theory that the oxygen liberated in photosynthesis is derived from the carbon dioxide. If the equation for photosynthesis in Section 9.5 is correct, then at least some of the oxygen released must come from the CO_2. If, however, Van Niel's theory is correct, the equation for photosynthesis would have to be rewritten:

$$6CO_2 + 12H_2O \longrightarrow C_6H_{12}O_6 + 6H_2O + 6O_2.$$

Faced with conflicting theories of this sort, scientists try to devise new experiments to test them. The usual method of procedure is to deduce from each theory a "fact" which must be so if the theory is sound. Then experiments must be devised to see which of the deduced "facts," if any, is actually a fact. In this case, the crucial experiments to test the two theories had to await the time when the growth of atomic research made the isotope ^{18}O available for general scientific use. You remember that isotopes are atoms whose weight differs but whose chemical properties do not. ^{18}O does not happen to be radioactive, but its presence in a compound can be measured. In 1941, a group of biochemists at the University of California allowed algal cells to carry on photosynthesis (1) in the presence of water which had been "tagged" with ^{18}O and (2) in the presence of CO_2 which had been tagged with ^{18}O. If the earlier theory was correct, they would have expected the ^{18}O atoms to appear in the liberated oxygen gas in the second experiment but not in the first. If Van Niel's theory was correct, however, the ^{18}O atoms should appear in the liberated oxygen in the first experiment but not in the second. The latter proved to be the case. Therefore, we now express the process of photosynthesis by the equation:

The use of ^{18}O to demonstrate that water is the source of the oxygen liberated in photosynthesis

$$6CO_2 + 12H_2O \longrightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O.$$

These experiments lent great support to Van Niel's idea that the role played by light in photosynthesis was the splitting of H_2O into hydrogen atoms and oxygen atoms. They gave no clue, however, as to the mecha-

nism by which the hydrogen atoms then reduced the carbon dioxide to glucose.

As you have seen (Section 9.6), it was through the work of Calvin and others that this process and the other steps in the sequence of dark reactions were worked out. You also remember that in the dark reactions, the reducing agent is not hydrogen itself, but $NADH_2$. In 1951 it was discovered that chloroplasts illuminated by light reduce NAD to $NADH_2$, which can then be used as the reducing agent in the dark reactions. But there still remained the problem of determining how $NADH_2$ could be manufactured from the hydrogen atoms liberated from H_2O.

Illuminated chloroplasts synthesize $NADH_2$

The problem has yet to be completely solved. However, Fig. 9.15 illustrates a mechanism which has received considerable experimental support. To understand this mechanism, it is necessary to understand the effect which light has upon chlorophyll.

Light energizes electrons in the chlorophyll molecule

When a solution of chlorophyll is placed in a beam of white light, it gives off light of a deep red hue. This phenomenon is called fluorescence. It can be demonstrated easily. A crude chlorophyll extract can be prepared by soaking grass leaves in ethyl alcohol. When placed in a beam of white light, the solution fluoresces.

The explanation of the phenomenon of fluorescence is that the energy of the absorbed light is transferred to an electron in the chlorophyll molecule. The electron is "excited" and raised to a higher energy level. In the chlorophyll solution, the electron drops back to its former energy level after a fleeting instant. In so doing, it gives up most of the energy that had excited it in the first place. The energy is released as red light.

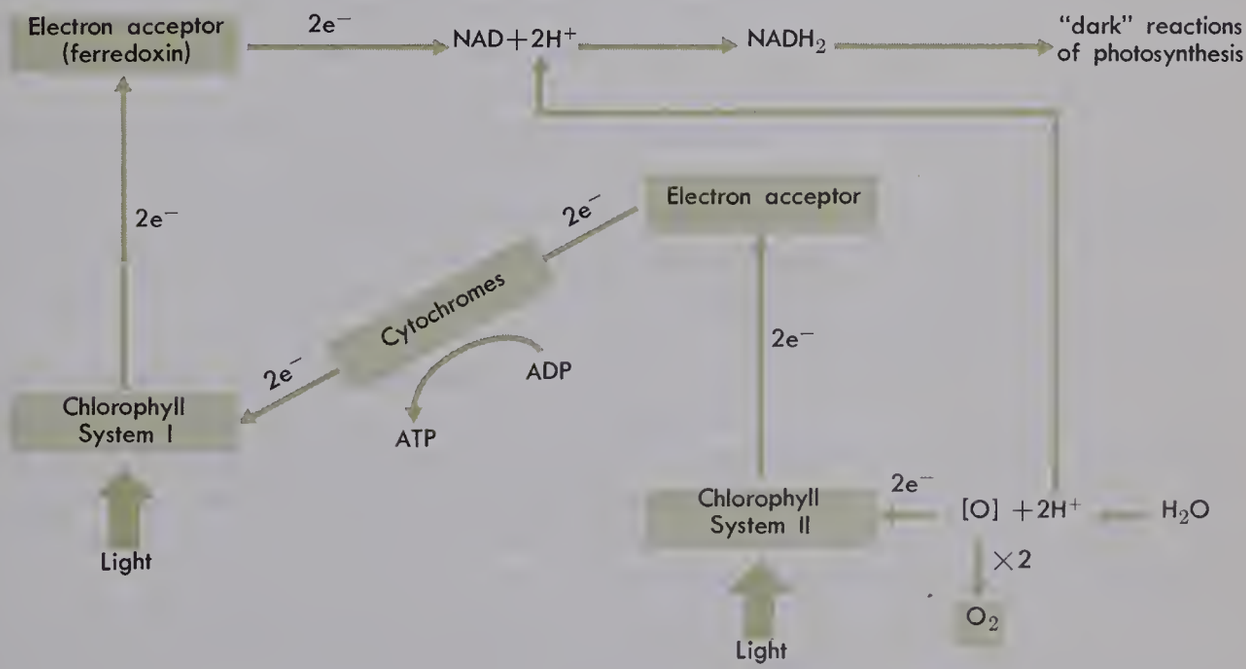


Fig. 9.15 Noncyclic photophosphorylation. The most important light-absorbing pigment in System I is chlorophyll *a*. In System II, most of the light is trapped by chlorophyll *b*. In the red, brown, and blue-green algae, the pigments responsible for their respective colors take the place of chlorophyll *b* in System II.

When *intact* chloroplasts are illuminated, very little fluorescence is observed. This could mean that many of the excited electrons are removed from the chlorophyll by some other substance before they can drop back.

Ferredoxin transfers electrons from chlorophyll to NAD

One such substance that has been identified is called ferredoxin. The excited electrons picked up by ferredoxin are sufficiently energetic to transfer to a molecule of NAD, thus reducing it. H^+ ions, present at all times because of the dissociation of water molecules, can then join with the electrons to give $NADH_2$. Finally, $NADH_2$ transfers its hydrogens to PGA in a dark reaction to form PGAL (Fig. 9.14).

You remember that plants and green algae have two kinds of chlorophyll in their chloroplasts. Chlorophyll *a* appears to play the dominant role in the reduction of ferredoxin. Other pigments may also participate in the trapping of light energy for this process, however; the complex of these pigments is called System I.

System I

To maintain the process of $NADH_2$ production, fresh electrons must be supplied to the chlorophyll molecules in System I to replace the electrons expelled in the reduction of ferredoxin. These fresh electrons come from OH^- ions. Note that in the dissociation of a water molecule, for every H^+ that joins with NAD, an OH^- is left behind (Fig. 9.15). The electron that gives the OH^- its charge is held tightly, and its removal requires a substantial amount of energy. The source of this energy, like that for the reduction of ferredoxin, is light. Light absorbed by pigments in System II causes the chlorophyll molecules to lose electrons and thus become positively charged. In this condition, they are a sufficiently powerful oxidizing agent to remove electrons from the OH^- ions. For every four OH^- ions which lose their electrons (become oxidized), two molecules of water and one molecule of oxygen are formed. The electrons are, in turn, raised to a high energy level as light continues to be absorbed by the pigments in System II.

Although chlorophyll *a* is probably responsible for the energizing and transport of electrons in both pigment systems, in System II it acquires much of its energy by transfer from chlorophyll *b* rather than by its own absorption of light. The difference in the absorption spectra of chlorophyll *a* and *b* (Fig. 9.3) increases the range of wavelengths useful in photosynthesis.

System II

Chlorophyll *b* is found only in plants and green algae. The red, brown, and blue-green algae have chlorophyll *a* plus other pigments which give them their respective colors. These other pigments are used in System II, and by transferring their absorbed energy to chlorophyll *a* they markedly extend the range of wavelengths that can be used for photosynthesis by these algae.

The pathway of electrons from System II to System I is not yet entirely clear. At least part of the journey, however, involves cytochrome enzymes in the chloroplasts. As the electrons pass through these cytochrome enzymes, some ATP is manufactured. Then the electrons are further energized during the absorption of light by System I and $NADH_2$ is formed. This process is called **noncyclic photophosphorylation.** It is noncyclic

Noncyclic photophosphorylation

because electrons must be continually supplied by the decomposition of water. It is a photophosphorylation because light energy results in the production of ATP. Surely, the pigments of System I and II as well as the cytochrome enzymes and the special electron acceptors must be properly oriented with respect to one another so that the flow of electrons can proceed with maximum efficiency. Although the details have yet to be worked out, it is likely that these molecules *are* precisely arranged in the thylakoids of the chloroplasts. Perhaps each **quantasome** (see Section 9.3) represents highly organized aggregates of the molecules necessary to carry out all the steps in the light reactions.

At first glance, it would seem as though noncyclic photophosphorylation accounted for all the substances needed to convert CO_2 to glucose, namely $NADH_2$ and ATP. Probably not enough ATP is produced by this method, however, to satisfy the needs of all the various dark reactions in photosynthesis.

One possibility for making up the deficit would be to use some of the $NADH_2$ as a reducing agent for the cytochromes in the mitochondria. You remember (Section 8.4) that in cellular respiration, $NADH_2$ donates its electrons (and hydrogen ions) to the cytochrome enzyme system, which eventually passes them on to oxygen. In the process, ATP is manufactured. This possibility seems somewhat unlikely, though, when we see that most green plant cells have a relatively small number of mitochondria compared with the number of chloroplasts they contain.

Cyclic photophosphorylation

This possibility was eliminated in 1954 when Dr. Daniel Arnon and his associates at the University of California showed that illuminated chloroplasts can manufacture substantial quantities of ATP even when isolated from other cell components (including mitochondria). Furthermore, this can be done even when no CO_2 is supplied to the chloroplasts and hence no dark reactions can occur. Even when the production of $NADH_2$ itself is stopped (by depriving the chloroplasts of NAD), the chloroplasts continue to produce large quantities of ATP as long as they are supplied with ADP, inorganic phosphate, and light. The energy of light is thus being converted directly into the chemical energy of ATP.

Dr. Arnon named this process **cyclic photophosphorylation.** A possible mechanism is shown in Fig. 9.16. Light absorbed by chlorophyll *a* energizes and expels electrons from the molecule so that they can be trapped by ferredoxin. This then passes the electrons to the cytochrome enzymes in the chloroplast. As they pass "down" the system, their energy is used to produce ATP (just as in the cytochrome enzymes of the mitochondria). The cytochromes then return the electrons to the chlorophyll molecule where they can once again be energized by absorbed light and passed through the cycle.

The chloroplast is a machine that converts the energy of light into the chemical energy stored in ATP

The process is truly cyclic because no outside source of electrons is required. The chlorophyll is simply trapping light energy and using it to energize its own electrons sufficiently to transfer them to some temporary electron acceptor in the chloroplast. On their route back to the chlorophyll molecule, they give up their acquired energy to form ATP. Thus chloro-

plasts can produce ATP as long as light shines upon them. This remarkable mechanism for converting light energy directly into the chemical energy of ATP more than makes up for the scanty production of ATP during the production of $NADH_2$ in noncyclic photophosphorylation. In fact, cyclic photophosphorylation probably provides (during daylight) the energy for many other energy-consuming reactions of the green plant cell. Not only the dark reactions of photosynthesis, but the energy for starch synthesis, protein synthesis, etc., can be supplied by the ATP produced in cyclic photophosphorylation.

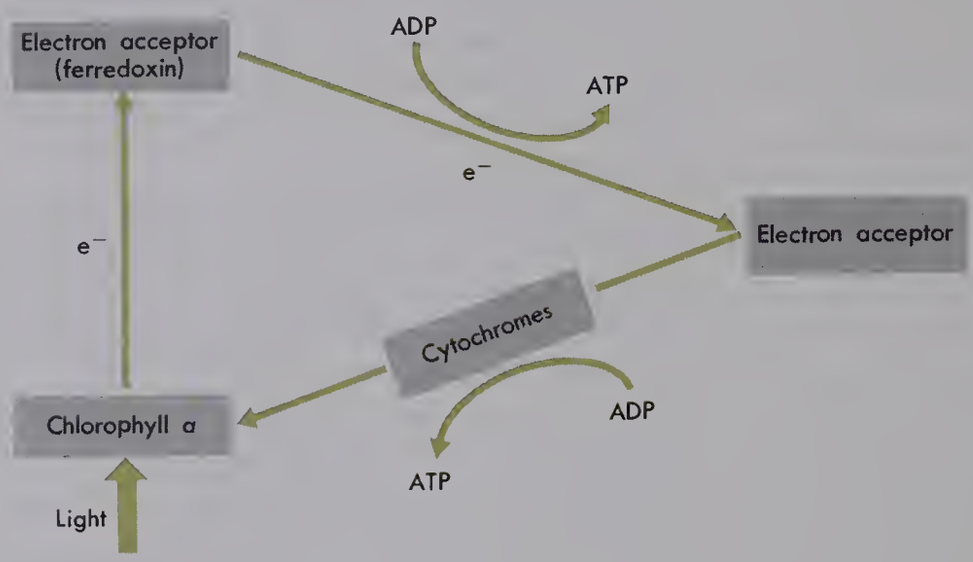


Fig. 9.16 Cyclic photophosphorylation.

In several respects, cyclic photophosphorylation resembles the second stage of noncyclic photophosphorylation, that is, the stage energized by the pigments in System I. Furthermore, there is some evidence that System II can absorb enough light to carry out noncyclic photophosphorylation without the help of System I. If this turns out to be the case, the role of System I will be simply the manufacture of ATP by cyclic photophosphorylation.

In any case, these light reactions (both noncyclic and cyclic) are thus the key to the ability of plants and algae to manufacture complex, energy-rich molecules from simple, energy-poor ones. As we have seen, heterotrophic organisms (such as ourselves) fulfill their energy requirements by converting the chemical energy of the glucose molecule into the chemical energy of ATP. The photosynthetic organisms can take care of their energy requirements by converting directly the energy of light into the chemical energy of ATP. In so doing, they produce organic molecules to supply themselves with energy in times of darkness. In producing a surplus of these organic molecules, they make possible the existence of all the heterotrophic organisms on this planet.

The light and dark reactions occur in different parts of the chloroplast

In the course of studying the light reactions and dark reactions in photosynthesis, methods were found to disrupt the chloroplasts and separate the pigment-containing thylakoids from the stroma. Although the

isolated thylakoids retained the ability to carry on both cyclic and noncyclic photophosphorylation, they could not convert CO_2 into carbohydrate. These dark reactions were readily carried out by the colorless stroma, however, when it was supplied with CO_2, $NADH_2$, and ATP.

As we saw in the case of the mitochondria, the efficiency of cell reactions cannot be properly understood without considering the physical arrangement of the enzymes that carry them out. When you consider that the green plant chloroplast can manufacture glucose from CO_2 and H_2O after only 30 seconds of illumuniation, you can appreciate that the many enzymes and pigments involved must be carefully arranged with respect to one another.

Photosynthesis and cellular respiration are complementary processes

In many ways, photosynthesis and cellular respiration are complementary processes. The transport of electrons lies at the heart of each. In photosynthesis, the energy of the sun is used to remove electrons from oxygen atoms in water molecules. These electrons are transferred to carbon atoms, forming covalent bonds between carbon and hydrogen. Carbon is far less electronegative than oxygen, and, consequently, energy is needed to make the transfer. Much of this energy is stored in the covalent bonds established. In this way photosynthesizing organisms form the food molecules upon which all organisms depend.

In cellular respiration, the electrons in food molecules are removed and allowed to return, step by step, to oxygen atoms. As they do so, they give back the energy originally stored by them, a substantial fraction of which is converted into the energy-rich bonds of ATP. In the last analysis, then, energy flow in all living things depends on the cyclic transfer of electrons between oxygen atoms and carbon atoms, accompanied by the breakdown (in photosynthesis) and synthesis (in cellular respiration) of water molecules.

CHEMOSYNTHESIS

9.8 THE CHEMOAUTOTROPHIC BACTERIA

A consideration of autotrophic nutrition would not be complete without examining the chemoautotrophic bacteria. Certain bacteria share the ability of chlorophyll-containing organisms to manufacture carbohydrates from inorganic raw materials, but do not need light energy to do this. Our study of photosynthesis has shown us that in itself there is nothing unique about this. The conversion of CO_2 into carbohydrate can go on in animal cells as well as plant cells. The same "dark" reactions are found to go on in the cells of the chemoautotrophic bacteria. The question is: what is the source of the energy that drives these energy-consuming reactions? How do the colorless chemoautotrophic bacteria manufacture sufficient ATP to produce glucose from CO_2?

The non-purple sulfur bacteria

In every case, chemoautotrophic bacteria have been found to secure the energy for these reactions by carrying out an oxidation of some substance present in their environment. Oxidation is an energy-yielding process, and the bacteria are able to harness this energy for the manufacture of carbohydrates. The chemoautotrophic **sulfur bacteria** oxidize H_2S in their surroundings (e.g. the water of sulfur springs) to produce energy:

$$2H_2S + O_2 \longrightarrow 2S + 2H_2O + \text{energy}.$$

This energy can then be used to reduce CO_2 to carbohydrate in the same manner as the purple photosynthetic sulfur bacteria do:

$$2H_2S + CO_2 \longrightarrow (CH_2O) + H_2O + 2S$$

(Section 9.7). Which organism do you suppose uses up more H_2S in the production of a molecule of glucose, the photosynthetic sulfur bacteria or the chemoautotrophic sulfur bacteria?

The iron bacteria

Another group of chemoautotrophic bacteria are the **iron bacteria.** (These are responsible for the brownish scale that forms inside the water tanks of flush toilets.) They complete the oxidation of partially oxidized iron compounds. In some way, they are able to couple the energy produced by this oxidation to the synthesis of carbohydrate.

The nitrifying bacteria

The **nitrifying bacteria** are also chemoautotrophic. They accomplish the oxidation of NH_3 (produced from proteins by the heterotrophic bacteria of decay) to nitrates. These oxidations provide the energy to drive the synthetic reactions of the bacteria. The nitrates produced as a by-product supply the nitrogen needs of plants. From them, plants produce amino acids and proteins. These proteins, in turn, supply the amino acids for heterotrophic organisms. While it is true that most organisms do not depend directly upon foods produced by the chemoautotrophic bacteria, here is an example where they do depend indirectly upon the metabolic activities of one group of these organisms. The role of the nitrifying bacteria in the over-all cycle of nitrogen on the earth will be considered again in Chapter 11.

EXERCISES AND PROBLEMS

1. The oxygen released in photosynthesis comes from which of the two raw materials used by the plant?
2. When green algae are illuminated simultaneously by two beams of single-wavelength light (about 45 nm apart at the red end of the spectrum), the amount of photosynthesis that takes place is markedly greater than when just a single wavelength of the same total intensity is used. Can you account for this? *Hint:* Refer to Fig. 9.3.
3. Life can be said to be fundamentally a matter of the transport of electrons between energy levels. Defend this view.
4. Distinguish between an autotroph and a heterotroph.
5. What is the most important reducing agent in living cells?

6. What color of light do green leaves absorb least well?
7. Do you think that the rate of photosynthesis would continue to increase indefinitely with increasing temperature? Explain.
8. Do you think that the rate of photosynthesis would continue to increase indefinitely with increasing carbon dioxide concentration? Explain.
9. When students perform the experiment illustrated in Fig. 9.9, starting with minimum light intensity, they often find that the rate of photosynthesis does not level off sharply at higher light intensities. What factor have they neglected to keep constant?
10. Students who have taken an extra-long time to carry out the experiment in Fig. 9.9 often find that the rate of photosynthesis begins to drop at high light intensities. Can you think of an explanation for this?
11. In two columns, contrast photosynthesis and respiration in as many ways as you can.
12. Do green plants need digestive enzymes? Explain.
13. How many electrons are removed from water molecules for each molecule of O_2 produced in photosynthesis?
14. How many electrons are needed for each molecule of CO_2 assimilated in the process of photosynthesis? How many $NADH_2$ molecules? How many ATP molecules?
15. How many ATP molecules are produced when 4 electrons pass from System II to System I? Relate this to the inadequacy of noncyclic photophosphorylation alone to meet the needs of the dark reactions.

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6. Lehninger, A. L., "How Cells Transform Energy," *Scientific American,* Offprint No. 91, September, 1961. Emphasizes the reciprocal relationship between photosynthesis and respiration.

10 ENERGY FLOW THROUGH THE BIOSPHERE

The Input of Energy

Ecosystem Productivity

Food Chains

Energy Flow through Food Chains

The Biomes

Fire

Plant Succession

10.1 THE INPUT OF ENERGY

In the preceding chapters we have examined how green plants store the energy of the sun in photosynthesis and how *all* organisms use this stored energy to live. We have examined these processes as they occur in an individual plant or animal. Now we shall turn our attention to the broader picture of how the sun's energy is trapped and used within entire communities of plants, animals, and microorganisms. Such a community, together with the nonliving features of its environment (e.g. air, water) with which it interacts, we shall call an **ecosystem.** The total of all the world's ecosystems (minus any overlaps between them) constitutes the **biosphere.** Our shift in emphasis from individuals to ecosystems will be like shifting one's attention from the way a single person earns and spends his money to the economics of a whole region or country.

Ecosystems

The biosphere

Solar energy

The energy that reaches the earth from the sun constitutes a small region in the spectrum of electromagnetic radiation (Fig. 10.1). It includes radiation with wavelengths between 400 and 700 nm (1 nm = 10^{-9} meter) to which the human eye is sensitive and which we thus call visible light.

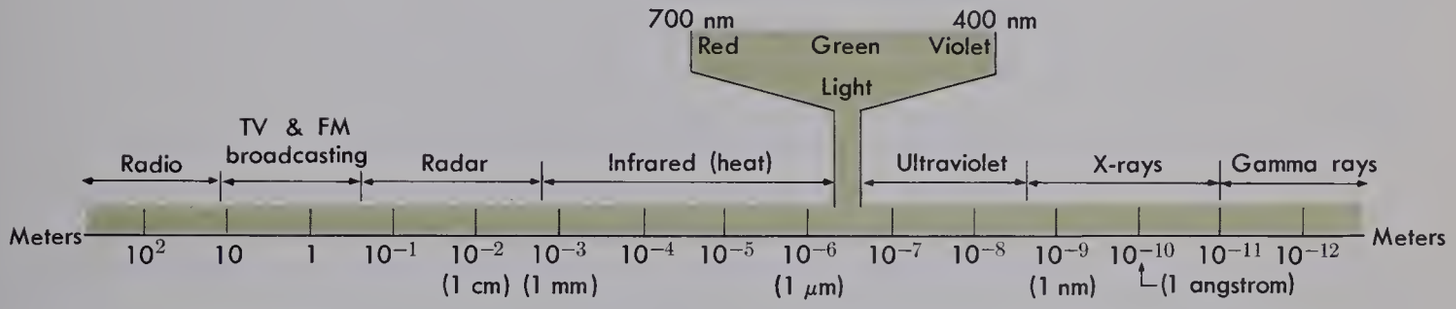


Fig. 10.1 The spectrum of electromagnetic radiation. The scale is logarithmic, every point representing a wavelength one tenth of that at the next point to the left.

The origin of seasons

It also includes a small amount of shorter wavelength ultraviolet light (responsible for tanning our skin) and longer wavelength infrared rays or heat. The intensity of radiation striking the earth varies with latitude and with the season of the year. The axis of the earth is tipped 23½° with respect to the plane in which the earth moves about the sun. For this reason, the northern hemisphere receives more than 12 hours of sunlight during the six months (approximately March 21 to September 23) when the axis of the earth is tipped toward the sun and less than 12 hours during the remaining months when the axis is tipped away (Fig. 10.2). The reverse situation occurs in the southern hemisphere. This phenomenon results in a net gain of solar radiation during one half of the year and a net loss during the other half, and hence is responsible for the seasons. The farther north (or south) from the equator one travels, the more pronounced the cooling of the earth in winter and the longer it takes for warm temperatures to return in the spring.

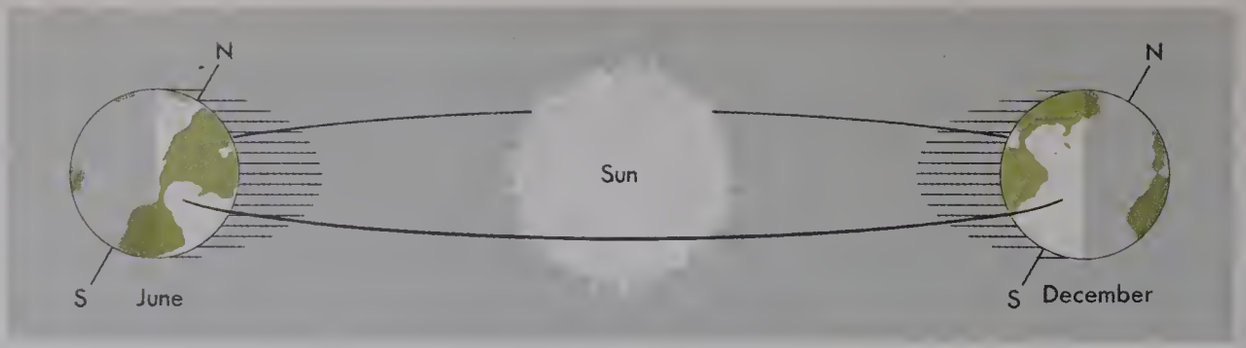


Fig. 10.2 The origin of seasons. Not only are the days shorter during winter but the intensity of the sunlight at the surface of the earth is diminished.

The amount of solar energy received diminishes with increasing latitude

Not only is there great seasonal *variation* in the amount of sunlight received at extreme latitudes, but the total amount received during the course of the year is less than that in the tropics. This is true even though the total number of hours that the sun is above the horizon is the same. The long days of summer do not compensate for the short days of winter. There are several reasons for this. The more extreme the latitude, the closer the sun is to the horizon, even at midday. Its rays, entering the atmosphere at an angle, must traverse a longer path before they reach the surface of the earth, and increased losses by absorption thus occur. Furthermore, the intensity of the light is reduced because a given amount of light shines on a larger area at extreme latitudes than it does at the equator (Fig. 10.2). The low angle at which the rays strike the earth also results in greater losses by reflection, thus further reducing the amount of energy available for photosynthesis.

The kcal/m² will be our unit of energy

Other things being equal (which they often are *not*), we would expect, then, that tropical regions would be more productive than temperate areas. They receive some 8000–10,000 kilocalories (kcal) of energy a day on each square meter of surface throughout the year. A kilocalorie is the amount of heat needed to warm 1 kilogram of water 1 degree Celsius (°C) or, more precisely, from 16.5 to 17.5°C. Because all of the light trapped in photosynthesis is ultimately released as heat, it makes sense to follow the flow of energy through the biosphere in units of heat.

Thanks to the long days of summer, temperate regions also receive some 8000–10,000 kcal/m²/day during the growing season. Thus we would expect their daily productivity during the summer months to equal that of the tropics. It may, in fact, be somewhat higher, although the few studies that have been made on the productivity of the tropics have yielded contradictory data.

10.2 ECOSYSTEM PRODUCTIVITY

How efficient is photosynthesis in converting the energy of the sun into the chemical energy stored in food molecules? One way to answer this question is to collect and weigh the amount of plant material produced by

Estimating productivity by harvesting

1 m^2 of land over a given interval of time. One gram of dried plant material (stems, leaves, etc.), which is largely carbohydrate, yields about 4.25 kcal of energy when burned (or respired).

A number of studies have been carried out using this approach and they demonstrate that an average temperate-climate forest stores approximately 5000 kcal/m^2 of energy during the course of a year. This is roughly equivalent to the production of 2½ lb of dry plant material for every square meter of forest. Considering the *total* amount of radiation received and correcting for the fraction of that usable in photosynthesis, the efficiency of conversion is approximately 1% (Fig. 10.3). This value would be somewhat higher if one included the fraction of trapped energy that has to be used by the plant to run its own metabolic activities, that is, consumed by the plant in cellular respiration. In order to arrive at this figure, it is necessary to measure the rate of respiration of the plants being studied.

UTILIZATION OF VISIBLE SUNLIGHT IN A CATTAIL MARSH

Photosynthesis	2.2%
Reflection	3.0
Evaporation (incl. transpiration), and heating of surroundings	94.8
Total	100.0%

Fig. 10.3 If one considers the *total* electromagnetic energy reaching the marsh, only about 1% is trapped by photosynthesis.

Estimating productivity by CO_2 analysis

This can be done by enclosing them in a transparent container and measuring the rate at which the CO_2 content of the surrounding air increases during the night because of respiration. Measuring the rate of CO_2 *decrease* during the day (because of a rate of photosynthesis greater than the rate of respiration) enables one to calculate the increase in organic matter, at least in terms of carbohydrate. The amount of trapped energy that plants ultimately lose to respiration varies markedly from type to type, but 50% is about average for forests. Whatever the figure, though, the quantity of greatest concern to us is **net productivity,** that is, the amount of energy *stored* by the plant community over a given period of time.

Net productivity

Desert plant communities store some 500 kcal/m^2/yr and thus average only 10% of the productivity of temperate forests. Estimates of the productivity of tropical forests vary widely. Some measurements have indicated productivities four to five times that of temperate forests, while others have yielded much lower values.

A great deal has been said in recent years about the potential of the oceans to assist in the feeding of the growing human population. Such measurements of productivity as have been made suggest that we may already be harvesting the oceans at a rate close to their maximum sustainable yield. The oceans are most productive where nutrients are readily available close to the surface. This is true of shallow, coastal waters and of certain areas, for example, off the coast of Peru, where upwelling ocean currents bring nutrients up to a level where sunlight penetrates sufficiently

Productivity of the oceans

to make photosynthesis possible. Although an estimate of average productivity for the oceans as a whole is very uncertain, it is probably in the range of 500–1000 kcal/m^2/yr, or not much better than the productivity of deserts. Even granting that the oceans cover three-fourths of the earth's surface, they probably account for no more than one-third to one-half of its productivity.

Agricultural productivity

Most human food comes directly or indirectly from agriculture. Under optimum conditions, the productivity of certain types of agriculture may be extraordinarily high. Fields of sugarcane may store 25,000 or more kcal/m^2/yr. Planting three crops a year of the new strains of rice that are part of the "green revolution" can produce similar rates of productivity. However, the edible portion of the plant, the rice grains, store only one-fourth of the net productivity of the plants. As for agriculture in temperate climates, the productivities range from 5000 to 12,000 kcal/m^2/yr, about the same as for mature forests in the same areas (Fig. 10.4).

Fig. 10.4 Estimated net productivity of certain natural and manmade ecosystems. The values are only approximations and are subject to marked fluctuations because of variations in temperature, fertility, and availability of water.

Estimated net productivity of certain ecosystems (in kilocalories/m^2/year)	
Temperate deciduous forest	5,000
Tropical rain forest	15,000
Tall-grass prairie	2,000
Desert	500
Coastal marsh	12,000
Ocean close to shore	2,500
Open ocean	800
Clear (oligotrophic) lake	800
Lake in advanced state of eutrophication	2,400
Silver Springs, Florida	8,800
Field of alfalfa (a legume)	15,000
Corn field, U.S.	4,500
Rice paddies, Japan	5,500
Sugar cane, Hawaii	25,000

Intensive agriculture requires the input of energy from several sources

Although intensively managed agricultural land is capable of productivities that exceed those of most natural plant communities, the differences are somewhat illusory. The input of energy in natural communities comes almost entirely from the sunlight that shines on them. This is not so for high-yield agriculture. In order to achieve the yields that hybrid strains of corn, rice, and wheat have made possible, there must be not only an input of sunlight but also inputs of fertilizer, irrigation water, pesticides, and the mechanical energy needed to plow and cultivate the soil as well as to harvest the crop. The manufacture of fertilizer and pesticides and their transportation and application take energy. This energy comes, in most cases, from the burning of fossil fuels—coal, oil, gasoline, and natural gas. All of these represent the stored productivity of ecosystems existing earlier in the earth's history. The pumping of irrigation water and the running of machinery for plowing, cultivating, and harvesting likewise depend upon the consumption of fossil energy. Therefore, a realistic figure

for the net productivity of agricultural land should take into account the number of kcal/m^2/yr of energy from fuel (fossil sunlight) that were expended in the process.

10.3 FOOD CHAINS

What happens to the net productivity of a plant community? Some is harvested by plant-eating animals, the herbivores. These include not only animals like deer and cattle but many small herbivores such as insects. Some of the net production of plants is consumed by organisms of decay, chiefly fungi and bacteria. In certain plant communities, a fraction of the net production is stored. In bogs, for example, much of the plant debris escapes decay and accumulates as peat. Such deposits gave rise in the past to coal.

In an immature forest, the total amount of organic matter increases yearly as the woody perennials increase in size. This, too, represents storage. When the forest becomes mature, however, the loss of organic matter by death and decay, when added to the loss by grazing, equals the net productivity. At this point, there is no further increase in biomass from year to year. The term **biomass** (or **standing crop**) is used to describe the total organic matter present in an ecosystem.

Biomass

When some of the net productivity of a plant community is eaten, the energy passes to a heterotroph, which depends on it for its very existence. Grasshoppers, for example, grow and carry on all their activities thanks to the energy stored in the plant food they consume. Herbivores, in turn, provide food for carnivorous animals. Grasshoppers, to take our example, may be consumed by toads. The process of transferring energy from creature to creature may continue. The toad may be eaten by a black snake, which may, in turn, be consumed by a hawk.

A food chain

Such a pathway of food consumption is called a **food chain.** All food chains start with an autotrophic organism, a photosynthesizing organism such as a green plant. These organisms are called **producers** because only they can manufacture food from inorganic raw materials. Any organism, such as a cow or grasshopper, that feeds directly upon a plant is an herbivore or **primary consumer** (Fig. 10.5). Carnivores, like the toad, that feed on herbivores are called **secondary consumers.** Carnivores, like the snake, that eat secondary consumers are **tertiary consumers,** and so on. Each level of consumption in a food chain is called a **trophic level.**

Trophic levels

In actually determining what eats what in a natural community, one soon realizes that the various food chains are interconnected. Most animals consume a varied diet and, in turn, provide food for a variety of other creatures that prey upon them. Thus the energy present in the net production of the producers passes into **food webs** of considerable complexity (Fig. 10.5).

Food webs

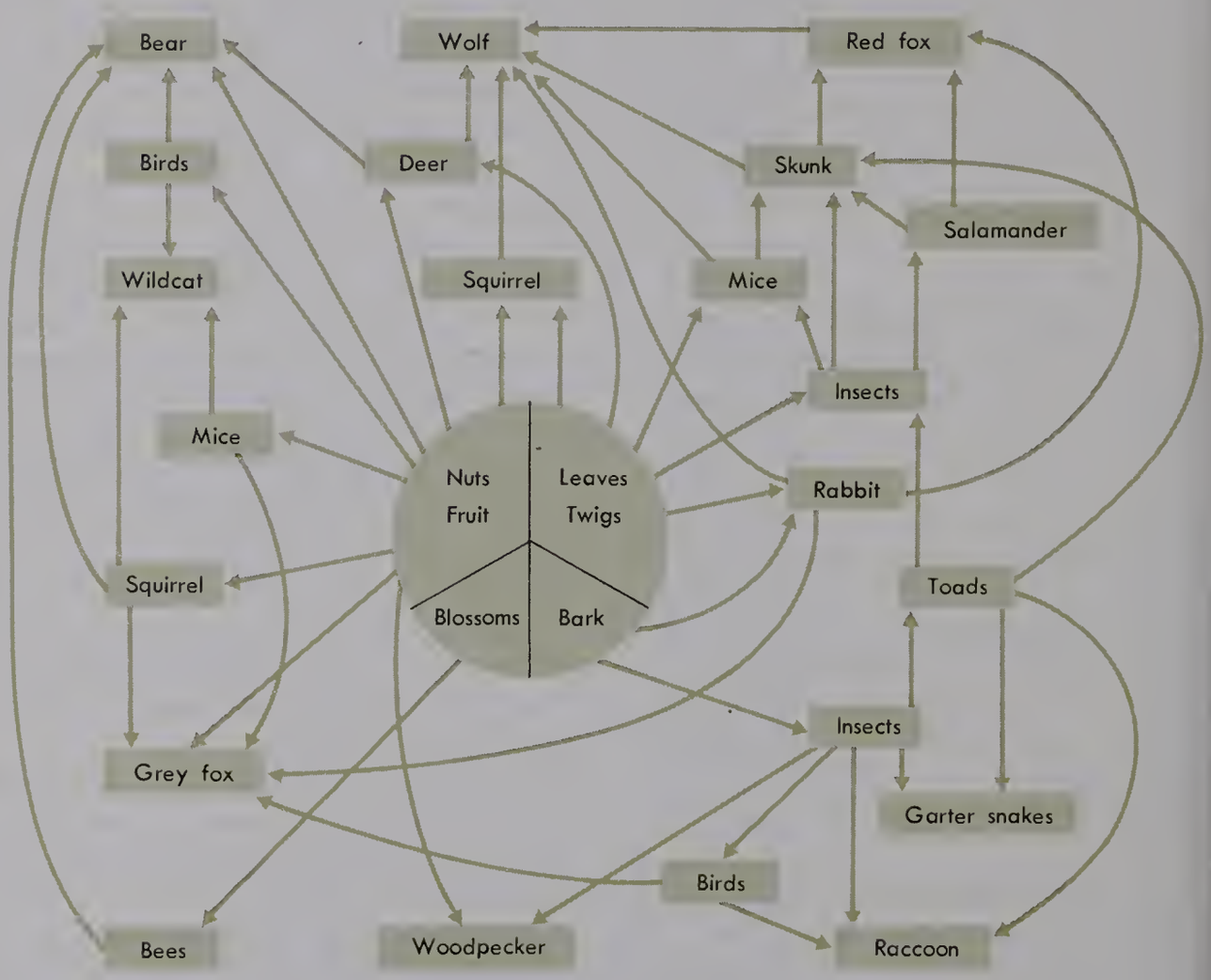
At each level of consumption in a food chain, some of the net production of that level is not consumed by the next higher level, but, after the death of the organisms, is decomposed by microorganisms of decay. These

Grass → grasshopper → frog → snake → hawk → bacteria of decay

In general,

autotrophs (producers) → herbivores (primary consumers) → carnivores (secondary, tertiary, etc. consumers.) → decomposers

(a)



(b)

Fig. 10.5 (a) Food chains. (b) A food web in a temperate deciduous forest. (Courtesy of Dr. V. E. Shelford.)

are chiefly fungi and certain bacteria, which are found in large numbers in soil and wherever else organic matter is present. They extract the energy remaining in the organic matter and, in doing so, release the inorganic products of its degradation (e.g. CO_2, NH_3) back into the surroundings. We have seen that the flow of energy through the biosphere is one-way: from the sun to the producers, then to the consumers, and finally to the organisms of decay. However, the materials out of which living things are

Matter is recycled; energy is not

built and which store the sun's energy must be recycled if the system is to continue operating. It is through the activity of the organisms of decay —working at every link in food chains—that much of this recycling is made possible. We shall examine in Chapter 11 some of the special roles that organisms of decay play in the recycling of matter.

10.4 ENERGY FLOW THROUGH FOOD CHAINS

The transfer of food from one trophic level to the next entails substantial losses of energy

How efficient is the conversion of the net production of one trophic level to the net production of the next? It is certainly far from 100%, although in some organisms, such as carefully bred varieties of poultry, the percentage of conversion may be remarkably high. Broilers can gain about half a pound of live weight for every pound of food ingested. (Since the water content of the two is not the same, the efficiency is actually less than the apparent 50%.)

It is unlikely that efficiencies of conversion much greater than this will ever be possible, because much of the energy consumed by an organism must be used to keep it alive and cannot be stored as net productivity. This energy is transferred by cellular respiration into the energy of ATP. As such it becomes available to (1) drive the metabolic activities that convert some of the ingested food molecules into more chicken, and (2) enable the chicken to move about, and maintain homeostasis, including keeping warm. In fact, the final form of energy resulting from all the metabolic activities of the chicken is heat. Thus it is no accident that the high conversion efficiencies achieved with these birds are possible only when they are reared in relative confinement and warm temperatures. The more physical activity an organism engages in, the smaller will be the percentage of its food used in growth, that is, contributed to net productivity.

Odum's Silver Springs study

A few attempts have been made to measure the flow of energy through food chains. One of the most elaborate of these was carried out by H. T. Odum on a river ecosystem, Silver Springs, in Florida. He found that the net production of the producers was 8833 kcal/m^2/yr (Fig. 10.6). Much of this material (5465 kcal) became plant debris that was decayed by decomposers or carried downstream out of the system. Herbivores consumed 3368 kcal/m^2/yr. Over half (1890 kcal) of this was lost, chiefly through cellular respiration. Thus the net productivity of the herbivores was 1478 kcal/m^2/yr. This represented 17% of the net productivity of the producers. Some of the primary consumers died and their remains decayed there or were transported downstream. Only 383 kcal/m^2/yr were consumed by secondary consumers. Of this, 316 kcal were used in their respiration, leaving only 67 kcal/m^2/yr of net productivity at this trophic level. This is only 4½% of the net productivity of the prior level. Such a low conversion efficiency is characteristic of carnivores, which must expend a high percentage of their energy in seeking prey from which to secure more energy.

Carnivores are wasteful of energy

Of the net productivity of 67 kcal/m^2/yr at the level of secondary consumers (first carnivores), 46 kcal were subsequently lost by decay and

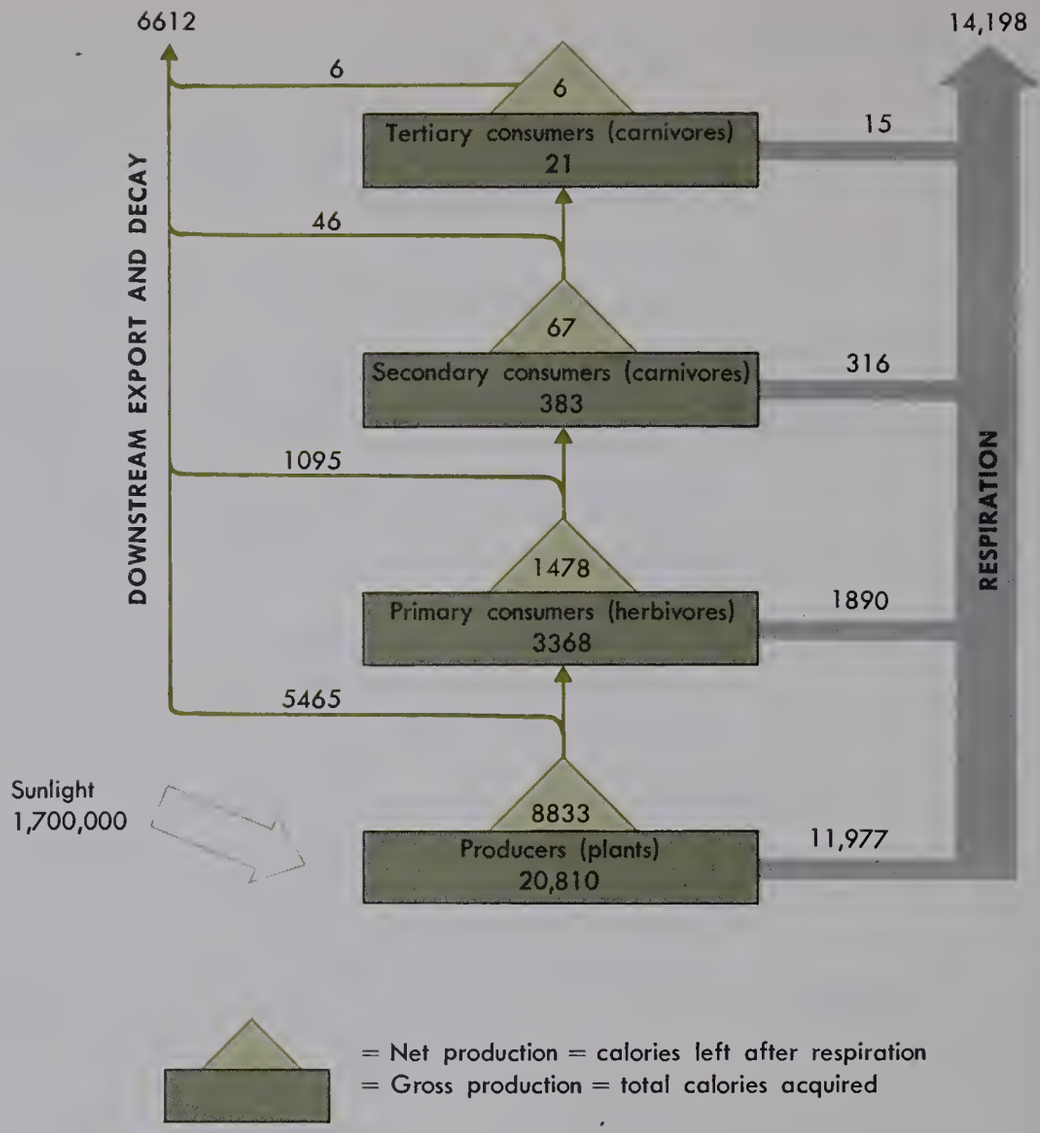


Fig. 10.6 The flow of energy through Silver Springs, Florida. The figures are given in kilocalories per square meter per year (kcal/m²/yr). (Based on data obtained by Howard T. Odum.)

The energy budget of a mature ecosystem is in balance

downstream transport. Only 21 kcal/m²/yr passed to tertiary consumers. Of this, they used 15 kcal in respiration, and had a net productivity of only 6 kcal/m²/yr (Fig. 10.6). Since there was no higher level of carnivores and no storage of energy (i.e. the biomass of the tertiary consumers did not increase from year to year), the net production was ultimately passed to decay organisms, either in the Silver Springs ecosystem or downstream. In contrast to the bog or young forests mentioned earlier, the Silver Springs ecosystem had no energy storage at any trophic level. To put it another way, neither the total biomass nor the biomass of any trophic level increased from year to year.

In the Silver Springs system, the efficiency of transfer of energy from one trophic level to the next varies from 17 to 4.5%. In fact, from similar studies in other ecosystems, we may use as a rule of thumb an average efficiency of transfer (net production at one level to net production at the next) of about 10%. Whatever the small variations that may occur from case to case, this rough approximation is of enormous significance. It explains, for example, why it costs more to buy a pound of beefsteak than a pound of corn. It took many pounds of the latter to produce one pound



Fig. 10.7 A pyramid of energy. At each link in the food chain, energy that was originally stored by the autotrophic grass plants is dissipated. What other relationships exist in such a food chain?

Why beef costs more than corn

of the former. Man includes both plant and animals in his diet. Whenever he eats meat, however, he is securing his energy third-hand. Its production has entailed substantial losses of energy. Of course, the situation is even worse for carnivores that prey upon other carnivores (e.g. when man eats a trout). Thus, at each link in a food chain a substantial portion of the sun's energy, originally trapped by the photosynthesizing autotroph, is dissipated back to the environment (ultimately as heat). We can conclude, then, that the total amount of energy stored in the bodies of a given population is dependent upon its trophic level. The total amount of energy present in a population of toads must necessarily be far less than that in the insects upon which they prey. The insects, in turn, possess only a fraction of the energy stored in the plants upon which they feed. This decrease in the total available energy at each higher trophic level is sometimes described as a **pyramid of energy.** In the food chain we discussed earlier, the autotrophic grasses provide the base of the pyramid. The carnivorous hawk occupies the narrow apex (Fig. 10.7). Using Odum's data

The pyramid of energy

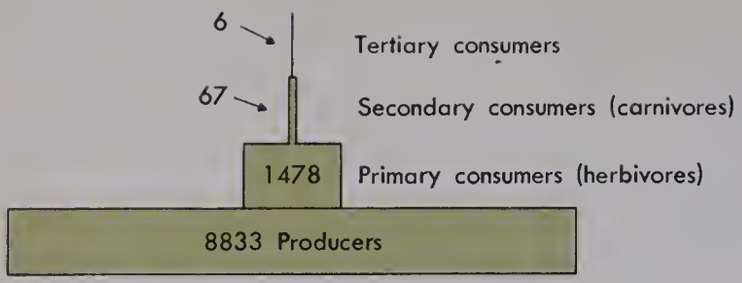


Fig. 10.8 Pyramid of energy in Silver Springs, Florida. The figures are given in kcal/m²/yr. (Based on data obtained by Howard T. Odum.)

Fig. 10.9 Pyramid of numbers in an acre of bluegrass. (Redrawn by permission from E. P. Odum, *Fundamentals of Ecology,* 2nd ed., W. B. Saunders Co., Philadelphia, Pa., 1959. Based on data acquired by Evans, Cain, and Walcott.)

		Number of individuals
Tertiary consumers	Carnivores (still larger)	3
Secondary consumers	Carnivores (larger)	354,904
Primary consumers	Herbivores (small)	708,624
Producers	Autotrophs	5,842,424

on net productivity at the various trophic levels in Silver Springs, we get the pyramid of energy shown in Fig. 10.8.

The pyramid of numbers

Another consequence of the ecological principles we have been discussing is that smaller animals are more numerous than larger ones. Figure 10.9 shows the **pyramid of numbers** resulting when a census of the populations of autotrophs, herbivores, and two trophic levels of carnivores is taken in an acre of grassland. The pyramid arises principally from the fact that each species is limited in its total biomass by its trophic level. If the size of the individuals is small, their numbers can be greater, and vice versa. Predators are *usually* larger than their prey, however, but being at a higher trophic level, their biomass must be smaller. Consequently, the number of individuals in the predator population is distinctly smaller than in the prey population.

10.5 THE BIOMES

If a resident of Pennsylvania travels to northern France, he may be impressed by the cultural differences he finds there but will probably feel that the general landscape is very much like home. A botanist would recognize that the species of trees and shrubs in northern France are in many cases not the same as those back in Pennsylvania but that the general types of plants and their growth habits are similar. Traveling south to the Mediterranean coast of France would be a different story. There the plants would present a landscape totally different from that of Pennsylvania (and one that would make a resident of southern California feel right at home). Not only would the plants be different, but a keen observer would find that the wild animals in the two regions differed as well. Such distinctive plant and animal communities are called **biomes.**

The exact number of different kinds of biomes on the earth cannot be given. This is simply because no area is completely homogeneous in its plant and animal life. If you like to emphasize the differences, you increase the number of biomes. If you are the type who overlooks differences in favor of uniformities, you choose a smaller number of biomes. For our purposes, however, we can consider that North America contains seven biomes.

Biomes are controlled by climate

A number of climatic features interact in the creation and maintenance of a biome. Where precipitation is moderately abundant (40 inches or more per year) and distributed fairly evenly throughout the year, the major determinant is temperature. Earlier in the chapter we examined the mechanism which creates lower average temperatures at increasing latitudes. Actually, it is not average temperature that is so important, but such limiting factors as (1) whether it ever freezes or (2) length of growing season.

Assuming adequate rainfall, we find four characteristic biomes as we proceed from a region of high average temperatures (the tropics) to a region of low (the Arctic).

1. The Tropical Rain Forest. In the western hemisphere, the tropical rain forest reaches its fullest development in the jungles of Central and South America. The trees are very tall and of a great variety of species. One rarely finds two trees of the same species growing close to one another. The vegetation is so dense that little light reaches the forest floor. Most of the plants are evergreen, not deciduous. The branches of the trees are festooned with vines and epiphytes. Epiphytes are plants that live perched on sturdier plants. Unlike vines, their roots do not reach the ground. Nor do they take nourishment from their host. Many orchids and many bromeliads (members of the pineapple family like Spanish "moss") are epiphytes.

Epiphytes

The diversity of animal life as well as plant life in the tropical rain forest is very great. A large proportion of the animal species, mammals and reptiles as well as birds, live in the forest trees.

The closest thing to a tropical rain forest biome in the continental United States is the little wooded "islands" found scattered through the Everglades in the southern tip of Florida (Fig. 10.10). Their existence depends on the fact that it never freezes and on protection from the fires that periodically sweep the Everglades. These fragments of subtropical rain forest are protected in the Everglades National Park.

2. The Temperate Deciduous Forest. The temperate deciduous forest biome of North America occupies the eastern half of the United States. It is characterized by hardwood trees (beech, maple, oak, hickory, etc.) which shed their leaves in the autumn. The number of different tree species here is far more limited than in the tropical rain forest biome, and large stands of a single species may often be found. As for animal life, deer, racoons, and salamanders are especially characteristic of this biome (Fig. 10.11).



Fig. 10.10 Vegetation in the Everglades of Florida. The abundance of vines and epiphytes is characteristic of the tropical rain forest biome. (Courtesy of William H. Amos.)



Fig. 10.11 Beeches and maples growing in the temperate deciduous forest of New Hampshire. (Courtesy of Eliot Porter.)



Fig. 10.12 The taiga, popularly known as the "spruce-moose" biome. (Courtesy of Lee E. Yeager.)

3. The Taiga. As one moves north into Canada, a new picture presents itself: a landscape dominated by conifers, especially spruces and firs (Fig. 10.12). This is the taiga, named after the similar biome found in the U.S.S.R. It is a land dotted with lakes and populated by bears, rodents (e.g. squirrels), birds, and moose. The latter is such a typical member that it has led some to call this the "spruce-moose" biome. During the cold winters, many of the mammals hibernate, while many of the birds migrate south.

4. The Tundra. Still farther north, the trees of the taiga become stunted by the harshness of the subarctic climate. Finally they disappear to reveal a land of bogs and lakes, a land so cold in winter that even the long days of the brief arctic summer are unable to thaw the permafrost beneath the surface layers of soil. Sphagnum moss, a wide variety of lichens, and some grasses and fast-growing annuals dominate the landscape during the short growing season (Fig. 10.13). Caribou feed upon this growth. So do vast numbers of insects. Swarms of migrating birds, especially waterfowl, invade the tundra in the summer to raise their young, feeding them with insects and a large variety of aquatic invertebrates and vertebrates. As the arctic summer draws to a close, the birds fly south, and all but a few of the permanent residents, in one way or another, prepare themselves to spend the winter in a dormant condition.



Fig. 10.13 Caribou grazing in the tundra of northern Canada. (Courtesy of Fritz Goro, *Life.*)

Effect of altitude on biomes

There is another way to visit the different biomes which does not require traveling hundreds of miles. Temperature is the major influence in establishing the four biomes discussed and, as we have seen, temperature varies with altitude as well as latitude. A trip up New Hampshire's Mt. Washington on the famous cog railway will, in a short time, enable one to pass from a temperate, deciduous, hardwood forest in the valley, through a region dominated by spruces, up into an alpine, lichen- and moss-dominated region near the summit. While the physical and biotic features of these zones are not identical with the biomes established by latitude, there are many close parallels (Fig. 10.14). As a rule, a climb of 1000 ft is roughly equivalent, in changed flora and fauna, to a trip northward of some 600 miles.

Rainfall in the western United States is affected greatly by mountain ranges

Three other major biomes are found in North America: grassland (prairie or plains), desert, and the chaparral. The controlling factor in these three biomes is not so much temperature but the amount and distribution of rainfall. The prevailing winds in the western half of our country blow in from the Pacific laden with moisture. Each time this air rises up the western slopes of, successively, the Coast Ranges, the Sierras and Cascades, and finally the Rockies, it expands and is cooled. Its moisture condenses to rain, which drenches the mountain slopes beneath. When the air reaches the eastern slopes, it is relatively dry, and much less rain falls. How much falls and when determine whether the biome in these areas will be grasslands, desert, or chaparral.

5. Grasslands. The annual precipitation in the grasslands averages 20 inches per year. A large proportion of this falls in the growing season. This promotes a vigorous growth of grasses (Fig. 10.15) but, except along river valleys, is barely adequate for the growth of forests. The factor that probably tips the balance from forests to grass is fire. There is considerable evidence that fires—set both by lightning and by man—regularly swept the plains in earlier times. Thanks to their underground stems and buds, grasses are not harmed by the fires that destroy most shrubs and trees.

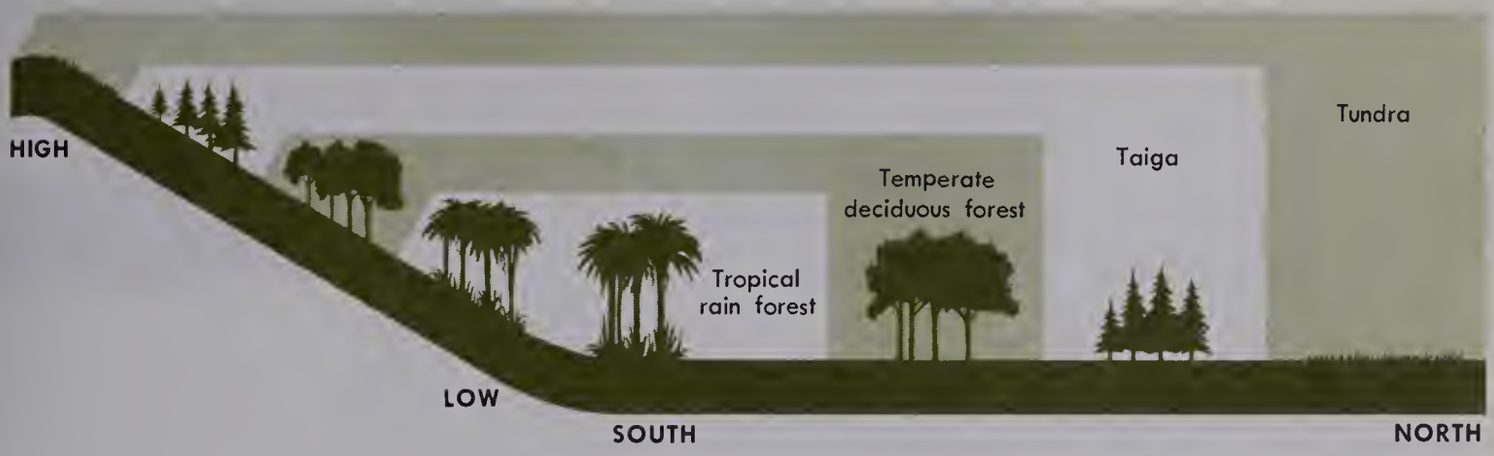


Fig. 10.14 The biomes change not only with latitude (right) but also with altitude (left).



Fig. 10.15 The grasslands biome. This plains area in Weld County, Colorado, has been only lightly grazed by cattle. (Courtesy of the U.S. Forest Service.)



Fig. 10.16 The desert biome in southern Arizona. (Courtesy of Dr. Dale A. Zimmerman and Ward's Natural Science Establishment, Inc., Rochester, N.Y.)

Because of its high productivity, most grassland in North America is now used for agriculture

The abundance of grass for fodder coupled with the lack of shelter from predators produces similar animal populations in grasslands throughout the world. The dominant vertebrates are swiftly moving, herbivorous ungulates. In this country, bison and antelope were conspicuous occupants of the grasslands before the coming of the white settlers. Now the level grasslands supply our nation with an abundance of corn, wheat, and other grains, while the hillier areas support our domesticated ungulates, cattle and sheep.

Rainfall

6. The Desert. Annual rainfall in the desert is less than 10 inches and, in some years, may be zero. Because of the extreme dryness of the desert, its colonization is limited to (a) plants such as cacti, sagebrush, and mesquite that have a number of adaptations by which they conserve water over long periods (Fig. 10.16) and (b) fast-growing annual plants whose seeds can germinate, develop to maturity, flower, and produce a new crop of seeds all within a few weeks following a rare, soaking rain. Most of the animals of the desert (mammals, lizards and snakes, insects, and even some birds) are adapted for burrowing to escape the scorching heat of the desert sun. Many of them limit their forays for food to the night hours.

Temperature

7. The Chaparral. The annual rainfall in the chaparral biome may reach 20 to 30 inches but almost all of this falls in the winter. Summers are very dry and all the plants—trees, shrubs, and grasses—are more or less dormant then. This biome is found in California. Similar biomes (with other names, e.g. scrub biome) are found around much of the Mediterranean, and along the southern coast of Australia. The trees of the chaparral are mostly oaks, both deciduous and evergreen. Scrub oaks and shrubs like manzanita and the California lilac (not a relative of the eastern lilac) form dense, evergreen thickets (Fig. 10.17). All of these plants are adapted to drought by such mechanisms as waxy, waterproof coatings on their leaves.



Fig. 10.17 Chaparral-clad foothills of the Sierra Nevada in California.

The chaparral has many plants brought from similar biomes elsewhere. Vineyards, olives, and figs flourish just as they do in their native Mediterranean biome. So, too, do eucalyptus trees transplanted from the equivalent biome in Australia.

10.6 FIRE

We usually think of fire as having only a harmful effect on biomes and work very hard to suppress it. But there is growing evidence that fire plays an important role in maintaining some biomes (or parts of biomes). We mentioned earlier that the grasslands biome probably requires periodic fires for its maintenance unless intensive grazing or mowing substitutes for them. Some of the subdivisions of the taiga and temperate deciduous forest biomes exist because of fire. The pine woods of our southeastern coastal region are an example of the latter. These pines are not damaged by ground fires and indeed their very existence seems to depend on them. When fires are excluded, deciduous hardwoods grow up under them, eventually replacing them. The transition may be hastened by fungi which thrive in the litter of the unburned forest floor and then move on to infect the pines. The ponderosa pine and redwood forests of our western mountains also appear to be dependent on fire to keep competing hardwoods at bay.

Some plant communities owe their very existence to periodic fires

The Everglades in Florida and the entire chaparral biome owe their very existence to fire. All the chaparral plants are adapted to fire and, after being burned, quickly sprout fresh growth (Fig. 10.18) which can support a larger population of herbivores (e.g. deer) than before. Tree-ring analyses indicate that fires swept through the chaparral in prehistoric times every few years.

The main reason for our aversion to forest fires is their great destructiveness, but this destructiveness occurs only with "crown" fires, that is, when the fire races through the tops of the trees. The vegetation destroyed by ground fires is quickly replaced, as we have seen. The irony of the matter is that by excluding fire, so much plant material accumulates close to the forest floor that when a ground fire does get started, it is so hot that it may quickly become a crown fire, destroying everything in its path. On the other hand, periodic ground fires keep the fuel level low. Dry chaparral plants are filled with resins and waxes and burn with great intensity. Almost every year enormously destructive fires occur in California. Perhaps the reintroduction of controlled, frequent fires will turn out to save more homes, watersheds, and ecosystem productivity than the present attempts to rigidly exclude fire in the chaparral.

Ground fires are often beneficial

When fire is excluded from an ecosystem where it has been common, changes in the plant community begin to take place. The changes in the southeast coastal pine forests, mentioned earlier, are an example. Any such long-term changes in a plant community are called plant successions.

10.7 PLANT SUCCESSION

Many plant communities are not self-sustaining. A field in the temperate deciduous forest biome will remain a field only as long as it is grazed or mowed regularly. If these activities cease, the grass plants begin to be replaced by other species. The elimination of an environmental factor such as mowing or fire tips the balance in favor of other species, and the newcomers may, in turn, by their own growth sow the seeds of their own

Mechanism of succession



Fig. 10.18 Chaparral in Southern California in the spring after a fire had swept the area the previous autumn. Many of the shrubs have already sent up fresh shoots from the ground line.

destruction. For example, the gray birch grows well only in sunny locations. The shade it casts prevents other gray birches from growing up beneath it but permits white pine seedlings to become established. When mature, white pines then cast so deep a shade that the gray birch is no longer able to survive in that area. By its growth, one population of plants alters the environment in a way unfavorable to it and favorable to some other plant population.

The process of plant succession begins just as soon as a land area capable of supporting plant life is formed. The exposure of rocks by a retreating glacier, the formation of beaches at the edge of oceans and lakes, and the gradual filling in of ponds all provide fresh terrain for plant colonization. Let us examine the plant succession which occurs on bare rock in the colder parts of the temperate deciduous forest biome.

Succession on bare rock

The first colonizers are the lichens and certain mosses. Acids secreted by the lichens attack the rock substrate and provide bits of soil. Additional soil particles may be formed by weathering or be blown to the rock from elsewhere and lodge in its crevices. Damage and decay to the lichen growth supplies some humus. Soon sufficient soil is present in the cracks for other mosses to become established. They produce fresh growth each season, the old growth decaying and quickly providing additional amounts of humus. Soon there is enough soil for grasses and, later, low shrubby growth such as blueberries. These, in turn, provide excellent cultural conditions for the seeds of such sun-loving, fast-growing trees as gray birch and poplar (quaking aspen). As we noted above, white pine soon replaces these short-lived species. In the dense shade of white pines, only shade-tolerant maple and beech seedlings thrive. When these large trees finally take over, the succession comes to an end. Maple and beech seedlings are able to develop under the conditions imposed by their parents, and the population becomes self-sustaining. It is known as a **climax** forest.

Succession in a bog

A similar process occurs as shallow ponds gradually fill in with soil washed in from the surrounding terrain and organic matter produced by underwater plants. As we walk from the edge of a poorly drained, boggy pond back into the forest, we pass through a series of zones that recreate in space the plant succession which has been occurring in time (Fig. 10.19). From the swamp loosestrife and arrowhead at the water's edge, past sphagnum moss and pitcher plants, then shrub-sized blueberries and poison sumac followed by black spruce and American larch and, finally, swamp maples and white pines, one passes concentric zones, each representing a later stage of plant succession as the soil has become firmer, dryer, and the shade denser.

Lumbering, grazing, farming, fires, and hurricanes interrupt the process of succession by removing the dominant plants in the community. Elimination of these factors sets the stage for a new succession to begin.

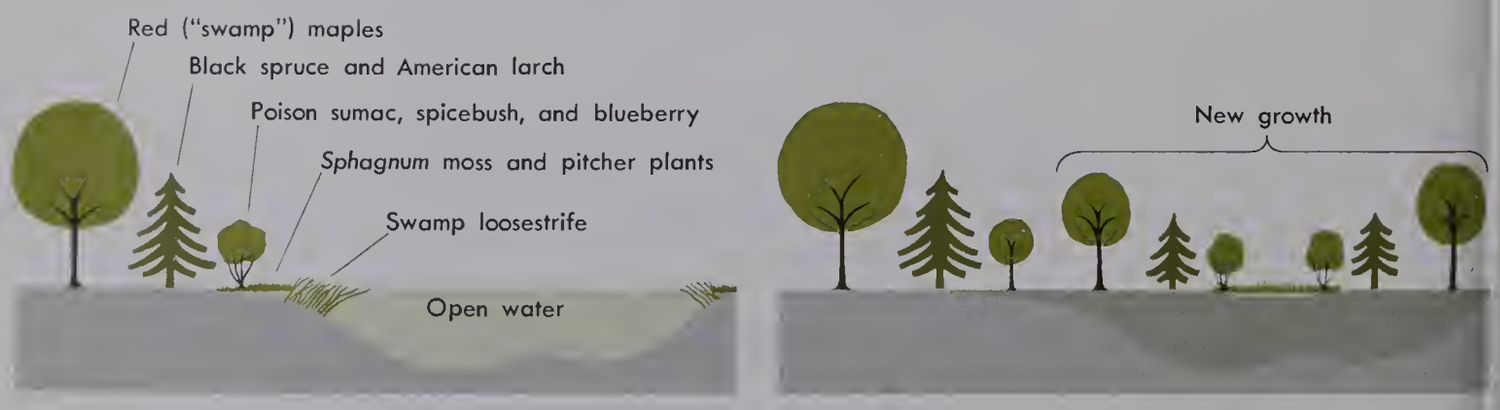


Fig. 10.19 Plant succession in a bog. Starting with the stems of the swamp loosestrife, which grow right out of the water, an increasingly thick mat of vegetation is formed as the years go by and the area of open water diminishes. Where water is still present underneath, the bog "quakes" when walked on.

The many abandoned farms in New England bear eloquent testimony to this. People often wonder why our pioneers built stone walls through the deep woods. The answer is they did not. The walls one sees in the woods today once marked the boundaries of fields and pastures, but when cultivation and grazing ceased, a **secondary succession** began. The grass of abandoned fields and pastures gave way to rank weeds and low shrubby growth. Soon gray birch, poplars, or cedars flourished. White pines, or, in sandy well-drained locations, oaks followed. If they are left alone, we will someday see once again a climax forest of maples and beeches.

Secondary succession

The colonization of bare rock, the filling in of a pond, and the secondary succession following the abandonment of a field each involve different species in the early stages of succession. In any given region, though, the species in the final, self-sustaining climax forest are the same. The tendency for all plant successions to end in the same climax community is called **convergence.**

Convergence

There are few parallels to plant succession in animal populations. An animal population may drastically alter the conditions of its environment so that it is no longer suitable for that population, but this is not inevitable. Nevertheless, succession in animal populations does occur simply as a consequence of succession in plant populations. As fields revert to woods, the kinds of birds, mammals, and invertebrates present change, too.

A climax community does not increase its biomass

In general, the process of plant succession is a reflection of the increasing efficiency of the community at intercepting the energy of the sun and converting it into chemical energy. As one stage of succession follows another, the biomass of the community increases. This is simply the outcome of the increasing amount of annual net productivity, i.e. calories, stored by the plant community. This, in turn, may provide the caloric support for an increasing population of consumers. When the climax forest is finally fully established, no further increases in net productivity occur. All of the annual net productivity of the plants is consumed by the heterotrophs, animals and microorganisms, of the community. The system comes into equilibrium and reaches peak efficiency at channeling the energy of the sun into all the food webs of the community.

EXERCISES AND PROBLEMS

1. Which of the following sources of energy depend ultimately on the energy of sunlight: (a) wood, (b) coal, (c) oil, (d) hydroelectric power, (e) atomic power?
2. What kind of organism ends every food chain?
3. What kind of organism starts every food chain?
4. In northern climates, how does each of the following manage to survive the winter: (a) frog, (b) maple tree, (c) hummingbird, (d) an annual grass such as "crabgrass," (e) a tulip, (f) a fern, (g) a bear, (h) a carrot plant, (i) a snake, (j) a lake trout, (k) a potato plant, (l) honeybees, (m) most other insects?
5. In what ways is a cactus well adapted to life in the desert?

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11

MATERIALS CYCLES

The Carbon Cycle

The Oxygen Cycle

The Nitrogen Cycle

Other Inorganic Nutrients

Water

Man's Use of Water

Soil

We have seen how the flow of energy through the biosphere is one-way. Radiant energy from the sun is intercepted by the biosphere. After passing through the transformations which keep all living things alive, it returns to outer space as heat. Thus there is no energy cycle.

The continued existence of life depends on recycling essential elements

The interception and use of energy by living organisms depends upon storing energy in chemical bonds and exploiting this energy when the bonds are broken. Living matter is built from a limited number, perhaps 24, of kinds of atoms. Some of these are abundant in the nonliving world. Some are very scarce (look back at Fig. 3.1). In either case, the continued existence of life on this planet for a period exceeding three billion years has been possible because of the existence of mechanisms by which these atoms can be used over and over again. Thus the atoms of life do go through cycles, and it is the nature of some of these cycles to which we now turn our attention.

11.1 THE CARBON CYCLE

Although carbon is a very rare element in the nonliving sector of the earth, it represents some 25% of living matter. The ability of carbon atoms to bond to each other provides the basis for the molecular diversity and molecular size without which life as we know it could not exist.

Organic compounds are formed by the reduction of CO_2

Outside of organic matter, carbon is found as the gas carbon dioxide and as carbonate rocks (limestone, coral). Autotrophs—chiefly green plants—take CO_2 and reduce it to organic compounds: carbohydrates, proteins, lipids, and others. Terrestrial producers get their CO_2 from the atmosphere, while aquatic producers use the bicarbonate that forms when CO_2 dissolves in water ($CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$). In Chapter 10, we traced the path of fixed carbon through food webs, all of which depend on it not only for their structure but also for their energy.

CO_2 is released by the oxidation of organic compounds

At each trophic level in a food web, carbon returns to the atmosphere or to water as a result of respiration. Plants, herbivores, and carnivores all respire and in so doing liberate CO_2 (Fig. 11.1). Much of the organic matter at each trophic level is not consumed by a higher trophic level but passes instead to the "final" trophic level, the decay organisms. This occurs as plants and animals or their parts (e.g. leaves in the autumn) die. Bacteria and fungi serve the absolutely vital function of unlocking carbon from corpses and debris that will no longer serve as food for other trophic levels. Through their metabolism, CO_2 is released and the cycle of carbon can begin again.

11.2 THE OXYGEN CYCLE

Oxygen is released in photosynthesis and consumed in cellular respiration

Molecular oxygen (O_2) represents 20% of the earth's atmosphere. This pool serves the needs of all terrestrial respiring organisms and, as it dissolves in water, the needs of aquatic organisms as well. In the process of respiration, oxygen serves as the final acceptor for electrons removed from the carbon atoms of food. The product is water. The cycle is completed in

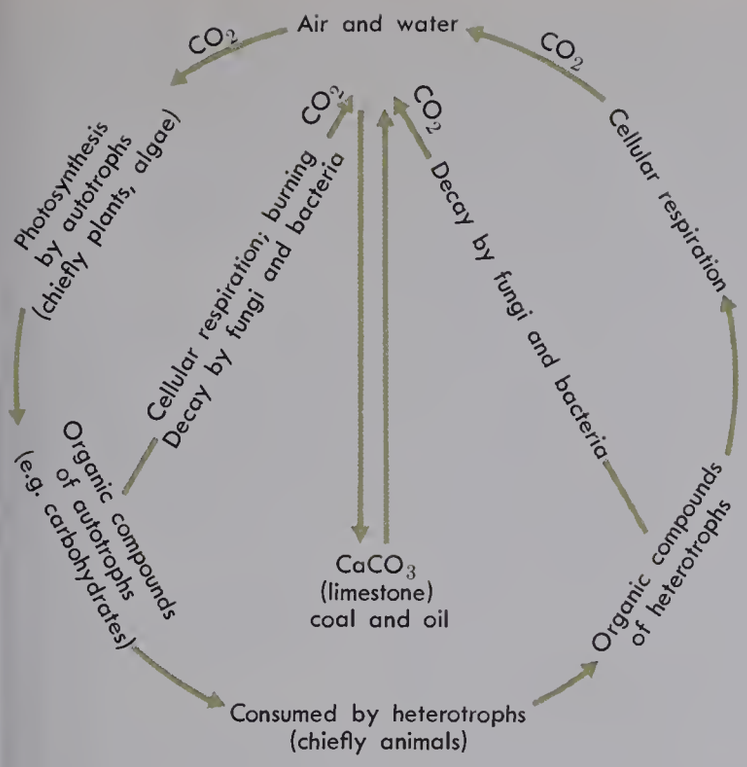
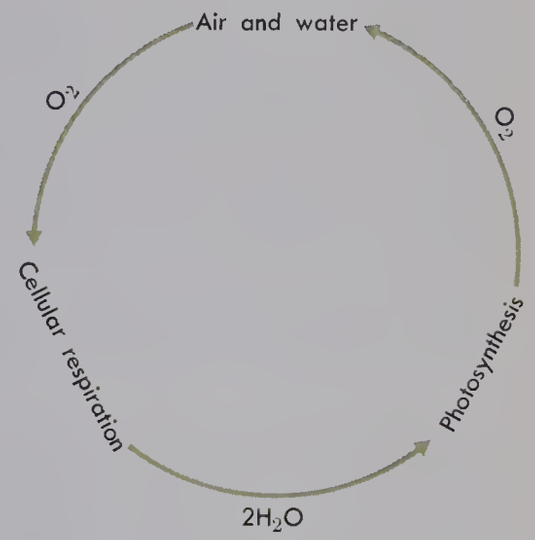


Fig. 11.1 The carbon cycle. The heterotrophic microorganisms of decay produce CO_2 by the respiration of organic molecules secured from the bodies of plants and the bodies and excreta of animals.

Fig. 11.2 The oxygen cycle.



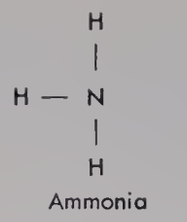
photosynthesis as the energy of light is used to strip electrons away from the oxygen atoms of water molecules. The electrons reduce the carbon atoms (of CO_2) to carbohydrate. Molecular oxygen is left over and the cycle is complete (Fig. 11.2).

The oxygen in the air was placed there by photosynthetic organisms

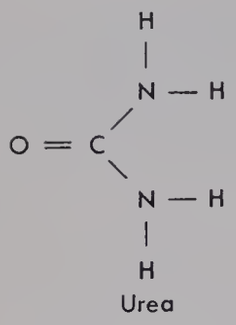
For every molecule of oxygen used in cellular respiration, a molecule of CO_2 is liberated. Conversely, for every molecule of CO_2 taken up in photosynthesis, a molecule of oxygen is given off. The study of minerals formed very early in the earth's history indicates that at one time there was no oxygen in the earth's atmosphere. With the evolution of photosynthesizing organisms, oxygen first appeared. Assuming the early development of a mature biosphere with no net productivity, that is, with respiration and photosynthesis in balance, we may well ask what accounts for the pool of oxygen present today. Each molecule of oxygen accumulated in the atmosphere must represent a carbon atom that was once reduced in photosynthesis but has since escaped oxidation. These are the carbon atoms sequestered away in coal and oil and other organic deposits. These are also the carbon atoms that make up the bodies of the worldwide living biomass and those dead portions of plants and animals that have so far escaped decay.

11.3 THE NITROGEN CYCLE

All living things require nitrogen atoms for the building of proteins, nucleic acids, and a variety of other essential organic molecules. Air, which is 79% nitrogen, serves as the reservoir of this substance. Despite the great



Ammonia



Urea

size of the nitrogen pool, it is often a limiting ingredient for living things, because most organisms cannot use nitrogen in its elemental form, that is, as the gas N_2. In order for plants to manufacture protein, they must secure nitrogen in "fixed" form, i.e. incorporated in compounds. The most commonly used form is nitrate ions, NO_3^-. Nevertheless, other substances such as ammonia, NH_3, and urea, $(NH_2)_2CO$, are used successfully, both in natural systems and as fertilizers in agriculture.

As we saw in Chapter 2, the nitrogen molecule, N_2, is quite inert. To break it apart so that its atoms can combine with other atoms requires the input of substantial amounts of energy. Three processes play an important part in the fixation of nitrogen in the biosphere. One of these is lightning. The enormous energy of lightning breaks N_2 molecules and enables them to combine with the oxygen in the air. (The process is analogous to that occurring in the internal combustion engine—see Section 12.10.) Nitrogen oxides are formed which dissolve in rain, forming nitrates. In this form they are carried to the earth (Fig. 11.3). Atmospheric fixation of nitrogen probably represents less than 10% of the total.

The need for nitrates in the manufacture of conventional explosives led to the development in Germany, on the eve of World War I, of an industrial process of nitrogen fixation. In this process hydrogen and nitrogen react to form ammonia, NH_3. In order for the reaction to proceed efficiently, it must be carried out at a high temperature (600°C), under enormous pressure, and in the presence of an inorganic catalyst. Today, most of the industrially fixed nitrogen is used as fertilizer. The initial product, ammonia, can be used directly as fertilizer. Most of it, however, is further processed to other common fertilizers such as urea and ammonium nitrate, NH_4NO_3.

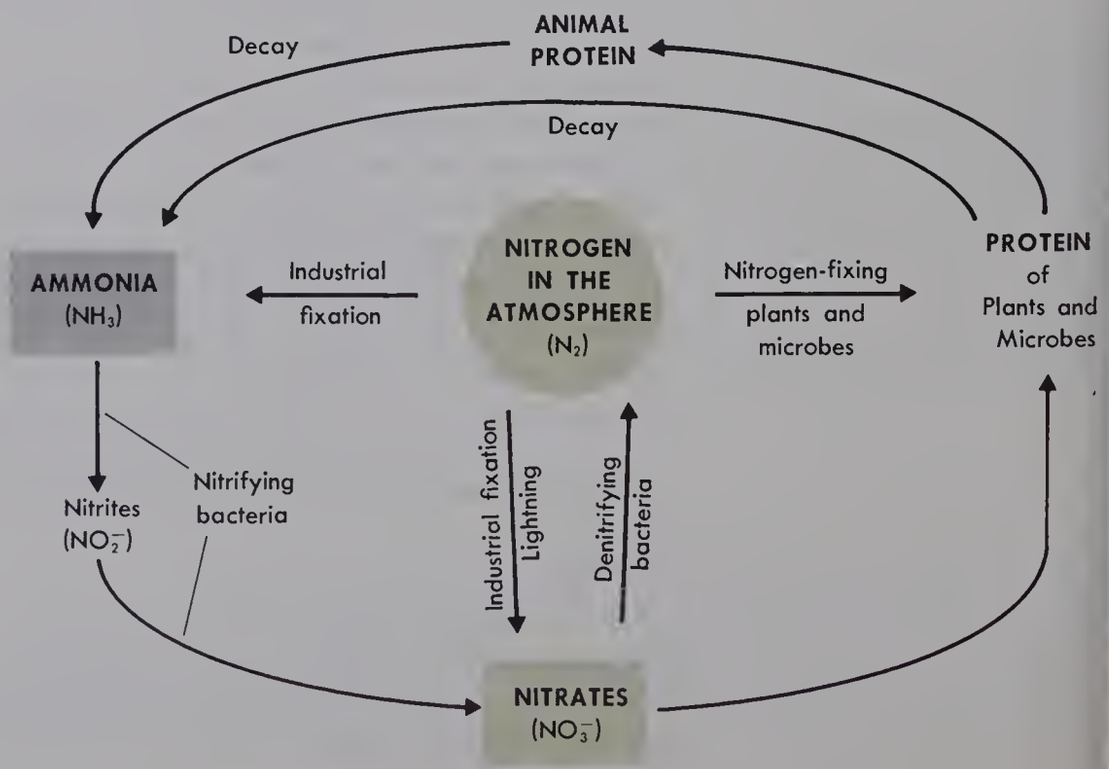


Fig. 11.3 The nitrogen cycle.

The growing needs of agriculture have led to an ever-increasing production of industrially fixed nitrogen. Perhaps as much as 40% of all the nitrogen fixation occurring today in the biosphere is accomplished industrially. This is really an extraordinary intrusion by man into the functioning of the biosphere. Whether such a perturbation of a natural cycle will work to our ultimate advantage is difficult to predict. Certainly our agricultural productivity depends upon the current high rates of nitrogen fixation. However, damaging side effects in our water supply are becoming apparent, as we saw in Chapter 2.

Symbiotic nitrogen fixation

Man's influence on the rate of nitrogen fixation is not limited to industrial activities. His widespread exploitation of symbiotic nitrogen fixation by legumes, especially alfalfa and soybeans, has greatly increased the worldwide rate of nitrogen fixation. There are microorganisms in addition to those found in legumes that can fix atmospheric nitrogen. Some of these are bacteria that live in loose association with plants other than legumes. Other nitrogen-fixing bacteria live free in the soil. Certain blue-green algae are also capable of fixing nitrogen and probably play an important role in maintaining the fertility of semi-aquatic environments like rice paddies.

Nonsymbiotic nitrogen fixation

The final product of nitrogen fixation is protein

Although the first stable product of nitrogen fixation is ammonia (see Section 2.5), this is quickly incorporated into protein and other nitrogen-containing organic compounds. For our purposes, then, nitrogen fixation leads to the incorporation of nitrogen into plant (and microbial) protein. Plants lacking the benefits of association with nitrogen fixers manufacture their proteins from nitrogen taken in from the soil (generally as nitrates).

The proteins manufactured by plants enter and pass through food webs just as carbohydrates do. At each trophic level, there are losses back to the environment, chiefly in excretions. The final beneficiaries of organic nitrogen compounds are the microorganisms of decay. Through their activities, the organic nitrogen molecules in excretions and corpses are broken down to ammonia.

Decay converts organic nitrogen compounds into ammonia

Ammonia can be taken up directly by plants—through their roots and, as demonstrated in some species, through their leaves. (When exposed to isotopically labeled ammonia gas, the leaves incorporate the label in their proteins.) However, most of the ammonia produced by decay is converted into nitrates. The conversion is accomplished in two steps. Bacteria of the genus *Nitrosomonas* oxidize NH_3 to nitrites (NO_2^-). Nitrites are then oxidized to nitrates (NO_3^-) by bacteria of the genus *Nitrobacter*. These two groups of bacteria are called nitrifying bacteria. Through their activities (which, being oxidations, supply them with all their energy needs), nitrogen is made easily available to the roots of plants.

Nitrification

Were the processes described above the complete story of the nitrogen cycle, we would be faced with a steady reduction in the pool of free, atmospheric nitrogen as it became fixed and began cycling through ecosystems (Fig. 11.3). Another process, denitrification, reduces nitrates to N_2, thus replenishing the atmosphere. Once again, bacteria are the agents involved. They live deep in the soil and in aquatic sediments where oxygen

Denitrification



Fig. 11.4 Measuring the cycling of materials in a forest in New Hampshire. The input of minerals into the valley is determined by measuring, with rain gauges, the amount of precipitation and its mineral content. The output from the valley is determined at this installation by measuring the discharge of water from the single stream that drains the valley and determining its mineral content. In recent years, the forest has been accumulating $SO_4^{=}$, NO_3^{-}, and K^{+}, while having a net loss of Na^{+} and silica. (Courtesy of USDA, Forest Service.)

is in short supply. They use nitrates as an alternative to oxygen for the final electron acceptor in their respiration. In so doing they close the nitrogen cycle. Whether their activities are keeping up with man's ever-increasing efficiency at promoting nitrogen fixation remains to be seen.

11.4 OTHER INORGANIC NUTRIENTS

Potassium, calcium, sodium (animals only), phosphate and, to a lesser extent, a variety of other ions (Fig. 6.1) are also necessary to life. Plants absorb these from soil water through their roots. Animals ingest inorganic ions in their food and water or, in the case of aquatic species, absorb them through their gills. In the case of phosphorus and sulfur, animals supply

their needs by ingesting phospholipids, nucleic acids, and proteins in their diet. All of these elements are returned to the soil and water by the action of decay bacteria on animal wastes and plant and animal bodies.

Over large areas and long spans of time, the natural recycling of inorganic nutrients provides adequately for the welfare of succeeding generations of living organisms. Under some conditions, however, local shortages may occur. This is particularly apt to happen when man practices intensive agriculture. Minerals are removed from the soil with the crops, but little or no decaying matter is returned to the soil to replenish the supply. Under these conditions, it is necessary to add fertilizers to the soil in order to maintain productivity. Nitrates and phosphates are especially apt to be depleted quickly, and the loss can be offset by the addition of such materials as "nitrate of soda" ($NaNO_3$) and "superphosphate," largely $Ca(H_2PO_4)_2$. These minerals and others like them are mined or manufactured synthetically for this purpose. Some fertilizer is made from slaughterhouse waste, and in using this, man is simply hastening and making more uniform a natural step in the recycling of minerals. Fertilizers that contain a source of nitrogen, phosphorus, and potassium are called "complete." The concentration of each nutrient is indicated by a number on the package. The first gives the percent by weight of nitrogen which is, of course, in some compound form. The actual compounds incorporating phosphorus and potassium atoms vary from one sample to the next, but by convention the concentration of these elements is expressed as if they were present as oxides, P_2O_5 and K_2O, respectively.

Fertilizers

Thus, 5-10-10 fertilizer, for example, contains 5% nitrogen (in compound form) and a weight of phosphorus and potassium atoms equivalent to 10% P_2O_5 and 10% K_2O. Limestone ($CaCO_3$) is frequently added to the soil, especially in the eastern part of our country, to counteract the natural tendency of the soil to become more acid. Fertilizers containing magnesium, manganese, copper, boron, zinc, and sulfate ions are also used in special circumstances.

11.5 WATER

On both a local and a global basis, water participates in the cycling of all the other materials needed by living things (Fig. 11.4). Every substance discussed earlier in this chapter cycles through the biosphere with some assistance from water. And, like all the other ingredients of the biosphere, water itself moves through cycles (Fig. 11.5). Its major pool is the oceans, which contain an estimated 97% of the earth's water. The remainder is fresh water in liquid, solid, and vapor forms. About 75% of the fresh water is immobilized as the ice of glaciers and the pack ice of the polar regions. It has been estimated that if all glacial ice melted, the sea level would rise some 250–500 feet.

The global distribution of water

Less than 1% of the earth's water exists as fresh, liquid water. Much of this is present as ground water in rock and soil layers beneath the surface of the earth. The remainder is the surface water of lakes and rivers. Most

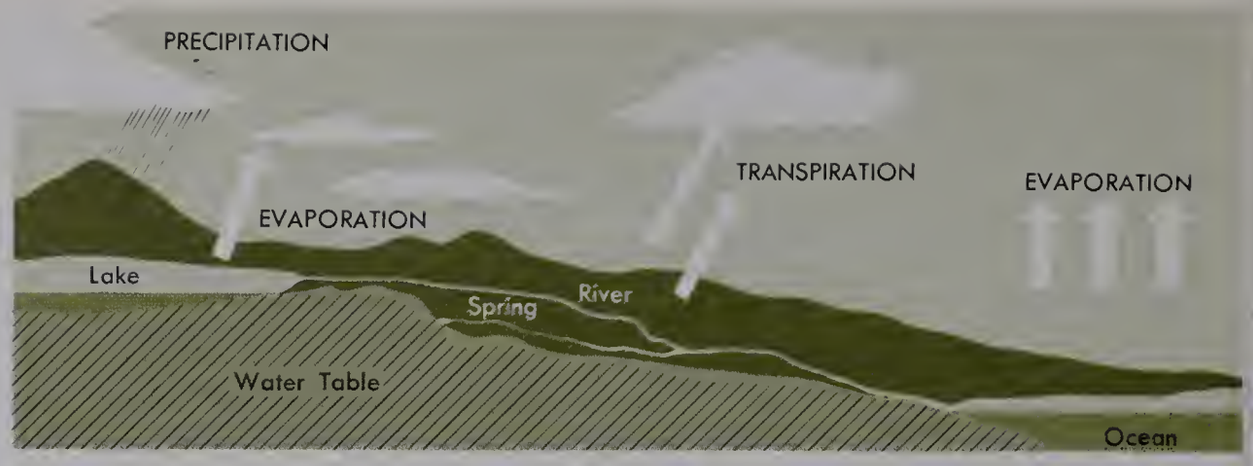


Fig. 11.5 The distribution of water. Evaporated sea water as it falls on land replaces the fresh water that flows constantly into the sea.

The water cycle

river water ultimately reaches the sea, bearing a load of salt, and mingles with the salt water of the sea. This continuing loss of fresh water is made up by the evaporation of sea water powered by the energy of the sun. Evaporation is simply low-temperature distillation and thus the salts are left behind. As a global average, an amount of sea water equivalent to a layer some 120 cm thick evaporates each year. When the water vapor is cooled, it condenses and returns to the earth as fresh water in the form of rain or snow. Of course it rains over the oceans as well, but the topography of the earth promotes precipitation and, as a consequence, land areas receive more precipitation than the oceans. The difference is made up by river flow back to the oceans.

Evaporation also occurs on land: from the surface of standing water, from exposed moist soil, and, especially, through the stomata of plants. Over a growing season, crops and forests transpire an amount of water equivalent to 50–80 cm of water and, for many land areas, this represents well over half of the total water lost to evaporation.

The water falling on earth first enters the surface layers of soil, from which it may pass to such bodies of surface water as ponds and streams. Where the necessary conditions are met, water may also pass down to the

The water table

water table. The water table is simply the upper surface of a subterranean zone of water-saturated soil and/or rock. Such a zone is known as an aquifer (Fig. 11.6). At certain places and times, the water table reaches the surface of the earth, forming springs. Many of these occur in water-filled depressions, adding to the flow of surface water in such "spring-fed" lakes and streams. Conversely, water in such bodies can move downward and thus replenish or enlarge the aquifer.

11.6 MAN'S USE OF WATER

A rapidly increasing proportion of the earth's fresh water is being used to meet the needs of a single species: man. The largest single use of water in

Irrigation

the United States is for the irrigation of crops. Almost as large is the use of water in various industrial processes. The manufacture of paper and the generating of electricity are two major industrial uses of water. Only about 10% of our water use is devoted to domestic needs.

A large percentage of irrigation water is temporarily "lost" by evaporation. In its other uses, however, water is not really consumed but rather it participates briefly in some process such as cooling or washing or flushing away wastes. Then it is released and theoretically could be repeatedly reused. The problem is that many water-using processes add materials to the water that make it unfit for other uses, especially for drinking and recreation.

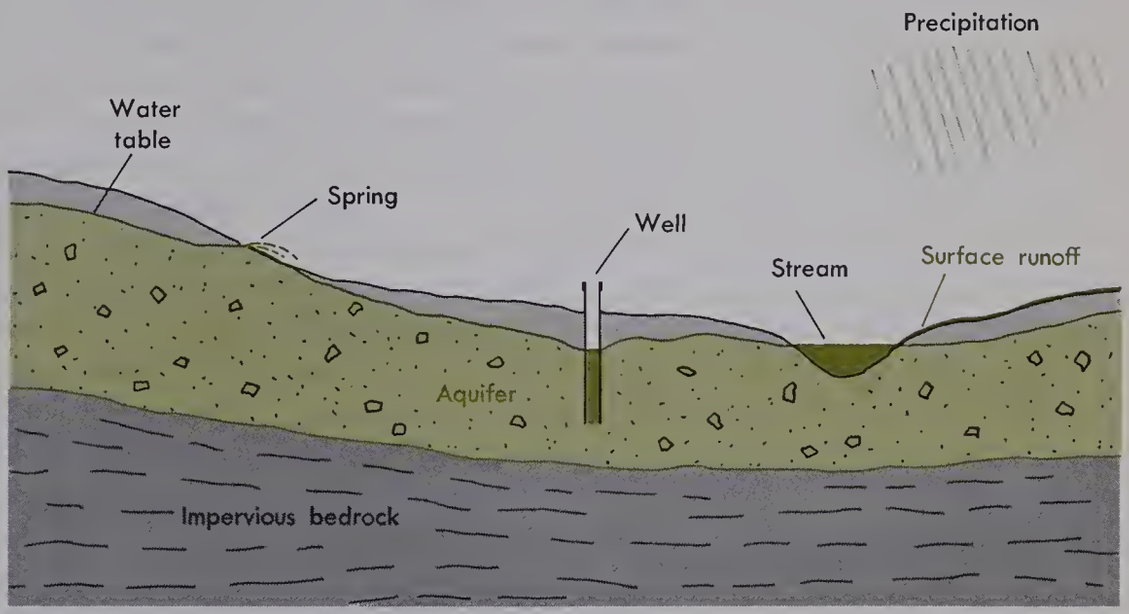


Fig. 11.6 Relationship between ground water and surface water. Pumping from streams and wells withdraws water from the aquifer. Water added to streams (and wells) replenishes the aquifer.

Industrial pollution

The wastes that industry adds to water are as varied as industry itself. They include a great variety of organic molecules that nourish aquatic bacteria. In breaking down these substances by cellular respiration, the bacteria are in effect purifying the water. If the system becomes overloaded, however, bacterial respiration may so deplete the dissolved oxygen content of the water that most "higher" forms of aquatic life (e.g. fish) cannot survive. Untreated domestic sewage also places great oxygen demands upon any water supply in which it is dumped.

Some industries also pollute water with inorganic materials and with heat. Mercury discharges by certain manufacturing processes have caused serious mercury pollution in parts of the United States, Canada, and Japan.

Eighty percent of the water used by industry is used simply for cooling. And the lion's share of this is used in the generation of electricity. Nuclear power is especially wasteful of heat. A nuclear generating plant

Thermal pollution

on the Connecticut River, and typical of those now in use, uses 372,000 gallons of cooling water each minute and discharges it back to the river 22°F warmer. While the ecological effects of such thermal "pollution" as yet seem innocuous, the projected growth of the nuclear generating industry suggests that caution be observed that no aquatic ecosystem becomes loaded with heat to the point where serious ecological consequences follow.

Domestic sewage, even when treated in conventional sewage-treatment plants, adds substantial quantities of nitrates and phosphates to our fresh water supply. The heavy use of fertilizer may also present a problem as spring rains and eroding fields start these same nutrients on their way into lakes and rivers. Phosphorus is generally the limiting nutrient in freshwater ecosystems for blue-green algae. That is to say, these algae are limited in their growth by the amount of phosphorus (usually present as phosphate) available, because blue-green algae can meet their *nitrogen* requirements by fixing atmospheric nitrogen (Section 11.3). Green algae, on the other hand, need both phosphates and nitrates in order to flourish. Adding one or both of these nutrients to fresh water may encourage such an explosive increase in the algal population that a "bloom" results (look back at Fig. 2.12). While algal blooms represent an increase in the productivity of the ecosystem, they are undesirable for several reasons. The respiratory needs of the algae may so deplete the oxygen content of the water during the night that fish suffocate. Furthermore, rapid algal growth may temporarily exhaust the nutrient supply, leading to a rapid dying off of the algae. The products of their decay may add such unpleasant tastes and odors to the water as to degrade its attractiveness. The increased productivity of lakes and streams brought about by nutrient enrichment is known as **eutrophication.** It has become a widespread problem in most countries with large populations and intensive agriculture.

Eutrophication

Meeting our increasing water needs

The world's water needs are increasing very rapidly. Already, fresh water is in such short supply in many parts of the world that man has had to turn to such expedients as (1) piping water hundreds of miles through aqueducts (e.g. in much of the U.S. southwest), (2) pumping "fossil" water out of underground water supplies, and (3) devising practical means for desalting sea water. None of these expedients promises to do more than simply allay the problem on a temporary and local basis. It seems quite clear that in order to meet our future needs for pure water, all water users will have to develop techniques by which, once used, the water can be returned purified and ready for reuse. In this way, fresh water could be intercepted and used repeatedly as it flows from the uplands to the sea.

11.7 SOIL

Plants "mine" the soil for minerals

For terrestrial ecosystems, soil is the point of entry of most materials into living matter. Through their roots plants absorb water, nitrates, phosphates, sulfates, potassium, copper, zinc, and other essential minerals. With these, they convert CO_2 (taken in through the leaves) into proteins,

carbohydrates, fats, nucleic acids, and the vitamins upon which they and all heterotrophs depend. Along with temperature and water, soil is a major determinant in the earth's productivity.

Soil horizons

A vertical section through the soil usually reveals several layers or horizons (Fig. 11.7). The top layer consists of partially decayed organic debris like leaves. Beneath this is the topsoil. This horizon is usually dark in color because of the decayed organic matter, humus, which has been incorporated in it from above. Productive agriculture depends upon a thick layer of topsoil. The reason is that humus gives the soil a loose texture that holds water and allows air to diffuse through it. Air is essential in order to meet the respiratory needs of plant roots, decay microorganisms, and all the other inhabitants of the soil.

Beneath the topsoil is often found a distinct horizon called the subsoil. This is usually lighter in color than topsoil and often contains a rich accumulation of inorganic nutrients.

Beneath the subsoil is a layer of weathered parent material. This represents the first steps in the chemical breakdown of rock into soil. The weathered parent material may, in turn, be underlaid by its own parent material or it may have been carried to its present location by wind, water, or glaciers. The chemical nature of the parent material, whether granite, limestone, or sandstone for example, has a great influence on the properties of the soil derived from it.

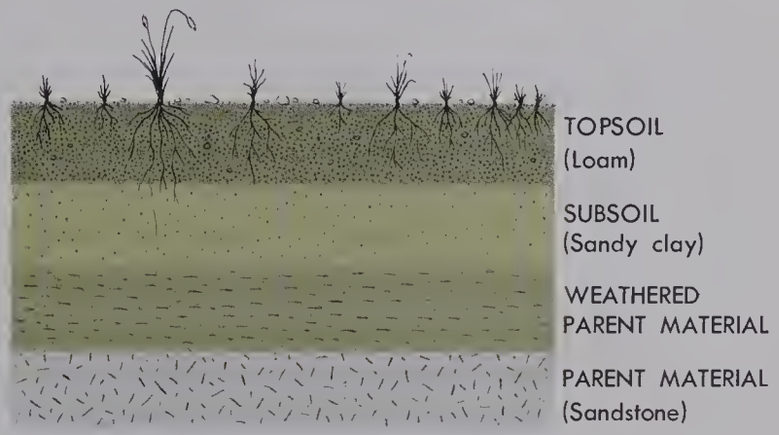


Fig. 11.7 A soil profile characteristic of the plains states. The rainfall is so light that soil minerals are seldom carried below the subsoil. As a result the fertility of this soil is easy to maintain.

Soil formation

The formation of soil is a dynamic process. It is the outcome of (1) the disintegration, through chemical action, of the parent material, (2) the formation (by microorganisms) and incorporation (by burrowing animals) of humus from above, and (3) the movement of minerals dissolved in the water percolating through it.

The amount of water falling on soil influences its properties and productivity tremendously. In humid areas (30–40 inches or more of precipitation per year), enough water falls on the soil so that much of it passes

on down to the water table. As it does, it carries minerals with it. Such soils tend to be acid, and of low and, if unattended, diminishing fertility. Only by regular fertilization and liming (to restore Ca^{++} and raise the pH) can productive agriculture be carried out in them. The soils in the eastern United States are of this sort.



In the plains states, the annual rainfall is sufficiently low (about 20 inches) that little or no rainwater ever percolates down to the water table. Calcium and other minerals are not carried below the reach of plant roots and consequently remain available for use. This keeps the pH and general fertility high. Except to the extent that minerals are lost when crops are removed, the minerals simply cycle from subsoil to plant parts to topsoil and back to subsoil. It is on account of the self-restoring fertility of the soils of the plains states that this region is known as the "breadbasket" of the nation.

In deserts, the rainfall is so low (10 inches per year or less) that water remains near the surface and is largely lost in evaporation. The salts it carries are left near the top of the soil. Their accumulation may make the soil so alkaline and so salty that most crops cannot grow. The situation is exacerbated in the Great Basin states (Utah and Nevada) because surface water flowing down from the mountains—bearing its load of dissolved salts—cannot flow on to the sea but simply flows out onto the valley floors and evaporates.

EXERCISES AND PROBLEMS

1. List four different kinds of bacteria that participate in the nitrogen cycle and tell specifically what each does.
2. What is the percentage of nitrogen by weight in a sample of 8-6-4 fertilizer?
3. Why do plants need nitrates and phosphates?
4. The topsoil layer tends to be much thicker in the grasslands than in the tropical rain forest. Can you think of a reason why this is so?

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12 GAS EXCHANGE IN PLANTS AND ANIMALS

Gas Exchange in Aquatic Organisms

Water versus Air

Gas Exchange in Terrestrial Plants

Gas Exchange in Terrestrial Animals

Mechanism of Breathing in Man

Air Pollution and Health

In order to carry on respiration, cells need a continuous supply of oxygen and a means of disposing of carbon dioxide. In order to carry on photosynthesis, green plant cells need a supply of carbon dioxide and a means of disposing of oxygen. Almost all cells are therefore involved in the problem of exchanging gases with the environment.

As you learned in Chapter 6, the environment of cells is the fluid which bathes them, the ECF. For gases to enter and leave cells, they must be dissolved in the ECF.

12.1 GAS EXCHANGE IN AQUATIC ORGANISMS

Most aquatic organisms depend on the oxygen that is dissolved *in water*

Among aquatic microorganisms such as the amoeba, the ECF is simply the surrounding water. The ratio of surface area to volume in the amoeba is sufficiently great that simple diffusion of the gases between the cell and the water is adequate to take care of its needs. As the amoeba respires, the consumption of oxygen by its mitochondria lowers the oxygen concentration within the cell. If the concentration of oxygen is greater in the water outside than within the cell, oxygen passes into the cell by diffusion. The oxygen concentration of the water is, in turn, maintained by: (a) diffusion of oxygen from the air into the water and (b) production of oxygen during photosynthesis by aquatic plants (Fig. 12.1).

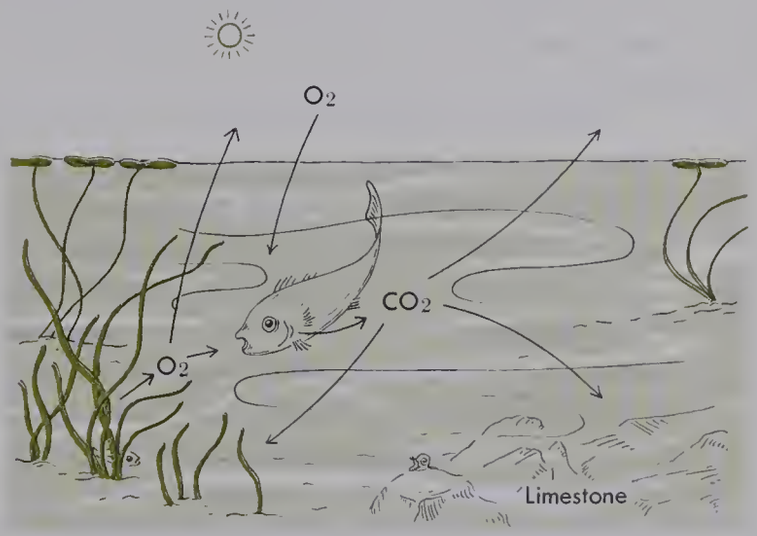


Fig. 12.1 Gas exchange in a fresh-water pond. Oxygen is released and carbon dioxide is consumed during photosynthesis. Carbon dioxide is released and oxygen is consumed in cellular respiration.

Carbon dioxide in the aquatic environment

While the amoeba is consuming oxygen, it is producing carbon dioxide. When the concentration of this gas within the cell becomes greater than its concentration in the surrounding water, it passes out of the cell by diffusion. The carbon dioxide concentration of the water is kept at reasonably low levels by three mechanisms: (1) Aquatic plants consume it in photosynthesis. (2) It diffuses into the air. (3) It is converted into bicarbonate and carbonate ions: $CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^=$. The latter are then converted into an insoluble mineral, limestone, which forms when carbonate ions and calcium ions are present together in the water: $Ca^{++} + CO_3^= \longrightarrow CaCO_3$ (Fig. 12.1).

Even among the smaller plants and animals that live in the water, the surface-to-volume ratio may be sufficiently large so that simple diffusion will take care of the needs of gas exchange. In the planarian, a flatworm found in fresh water, although many cells are buried beneath the surface, simple diffusion to and from the surface cells provides adequately for them. Perhaps the very flat, ribbonlike shape of the planarian is an adaptation to allow for the easier exchange of gases between the interior cells and the environment (Fig. 12.2).

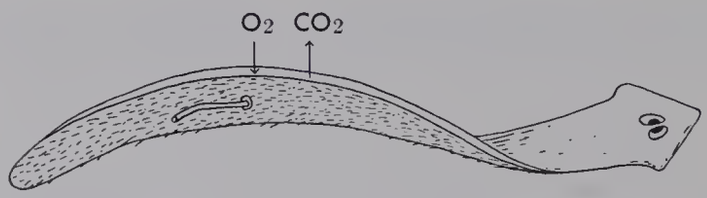


Fig. 12.2 Every cell in the thin planarian body is close enough to the surface for its gas-exchange needs to be satisfied by diffusion.

Above a certain size, organisms need special mechanisms to service the gas exchange needs of their internal organs

Among the larger aquatic animals, the ratio of surface to volume is smaller. Many cells, tissues, and organs of these animals are located too deep within the body to carry on adequate gas exchange with the environment by diffusion alone. An actively respiring interior cell cannot receive oxygen sufficiently rapidly if the oxygen must come to it by diffusion from cell to cell.

The most widespread solution to this problem is to bathe the interior cells in an ECF, blood or lymph, contained within the organism's body. This fluid bathes each cell and, in so doing, brings oxygen to it and removes carbon dioxide from it. It must also be able to pick up fresh supplies of oxygen from the surrounding water and to discharge its accumulation of carbon dioxide into it. This requires a specialized organ for **gas exchange.**

The gas exchange surface of gills is usually extended outward

Among the crustaceans, some aquatic insects, mollusks, and all fishes, the gas exchange organs are **gills.** Gills present a large surface to the water in which they are bathed. Oxygen present in the water diffuses into the cells of the gills. The gills are supplied with blood which transports oxygen from them to the interior tissues of the body. The same fluid picks up carbon dioxide from the interior tissues and transports it to the gills. From the gills the carbon dioxide passes into the water by diffusion. Figure 12.3 shows how this system works in a clam and in a fish. Note that there are three distinct and essential parts to the sytem: (1) A **circulating fluid** which transports the gases. (The fluid is kept moving with the help of a muscular pump, the heart.) (2) **Gills** which exchange gases between this circulating fluid (the internal environment) and the water (the external environment). (3) A mechanism for drawing a continuous **supply of oxygenated water** over the gills. In the clam, the rhythmic beating of the cilia which cover the gills draws water in through the incurrent siphon, through pores in the gills, and out through the excurrent siphon. In the fish, "yawn-

"Ventilation" of gills

ing" movements draw water into the mouth. The mouth is then closed. Contraction of the mouth cavity then forces the water through the arches which support the feathery gills. The water is finally expelled through the slits at the rear of the gill chamber (Fig. 12.3).

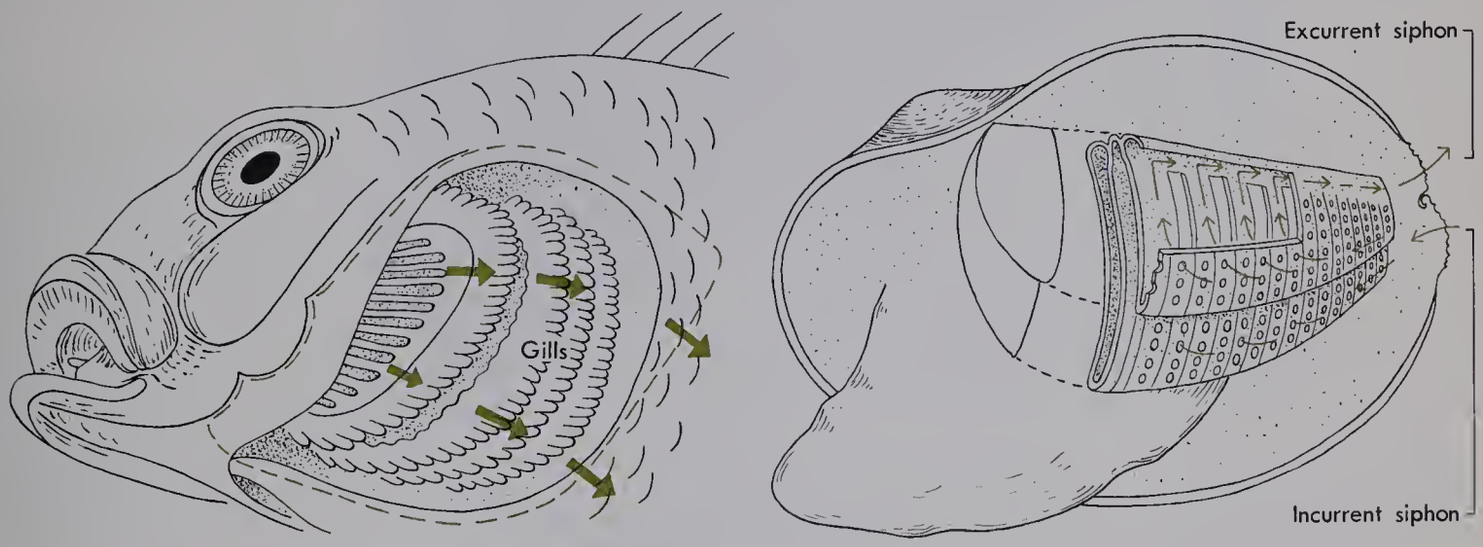


Fig. 12.3 Two gill-breathers: the fish and the clam. Part of the fish has been cut away to reveal the gills. In the clam the left shell and portions of the left gills have been cut away to show the pathway taken by the water.

12.2 WATER VERSUS AIR

In one respect, the problem of gas exchange is simpler for a multicellular, terrestrial organism than for an aquatic one. Oxygen represents about 21% of a given volume of air. Therefore, a terrestrial organism in the process of passing 100 ml of air over its gas-exchange organ has come in contact with 21 ml of oxygen. On the other hand, even in well-aerated water the oxygen concentration seldom exceeds one ml of gas in every 100 ml of water. Consequently, the aquatic organism must expose its gills to a relatively large flow of water to satisfy its oxygen needs. Perhaps this difference in the availability of oxygen in air and water is related to the fact that the only "warm-blooded" animals on earth are the air-breathing birds and mammals.

In most air breathers, the gas exchange surface is folded inward

On the other hand, terrestrial organisms are faced with the problem of keeping their gas-exchange organ moist even while it is exposed to dry air. As we discussed earlier, diffusion of gas into a cell only occurs from the ECF. The ECF may be only a thin film of moisture, but it must be there. The most general solution to this problem has been to have the gas-exchange organ extend inward into the interior of the organism. In this way, air passing into the gas-exchange organ can be pre-moistened by accessory membranes and its drying effect lessened.

GAS EXCHANGE IN TERRESTRIAL PLANTS

Each organ of the plant takes care of its own gas exchange needs

In plants no single gas-exchange organ supplies all the needs of the organism. Furthermore, there is very little transport of gases from one portion of the plant to another even though the plant has an elaborate fluid transport system, as we shall see in Chapter 14. For this reason, we find that each organ of the plant (root, stem, leaf) takes care of its own gas-exchange needs.

12.3 GAS EXCHANGE IN ROOTS AND STEMS

The gas-exchange needs of roots and stems are not great. Plant respiration usually proceeds at much lower rates than in animals. While photosynthesis does impose substantial gas-exchange demands, roots do not participate in this process. The oxygen needs of roots are met by diffusion of oxygen from the air into the spaces between the soil particles. From here the oxygen diffuses into the film of moisture that surrounds the soil particles and then into root hairs (extensions of the epidermal cells of the root tips) which are in contact with this film of moisture (Fig. 12.4). From the cytoplasm of the root hairs, the oxygen passes by diffusion to the other cells of the root. Carbon dioxide produced by the cells of the root leaves by diffusion in the reverse direction.

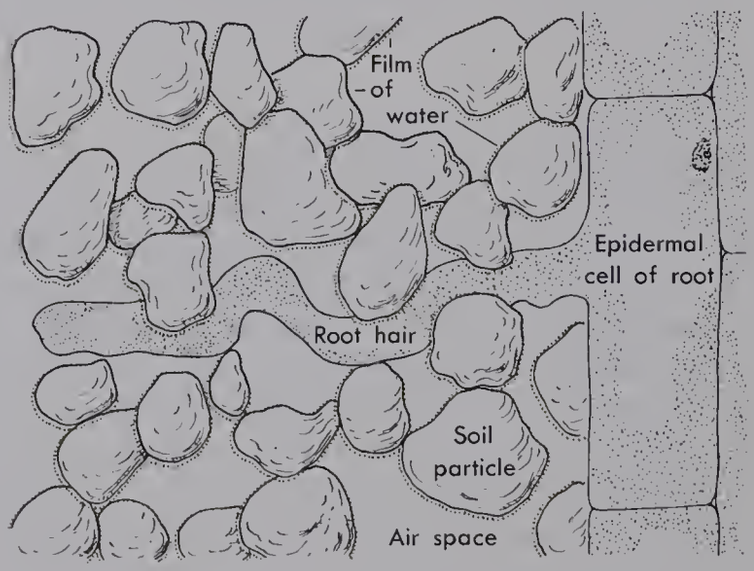


Fig. 12.4 The increased surface area provided by the root hairs aids not only in gas exchange but also in the absorption of water for use throughout the plant.

The thicker, older portions of roots do not have any root hairs. Here the roots are covered with a protective layer of dead cells, the cork. It contains many tiny openings, the **lenticels,** which permit gases to pass quickly to and from the soil and the inner, living cells of the root.

The roots of most plants will suffocate in waterlogged soil

Soil that is thoroughly soaked with water does not have any air between the soil particles. Most land plants will die if their roots are exposed to such waterlogged soil for a long period of time. Some plants



Fig. 12.5 Lenticels in the bark of a young stem. These openings enable the living tissues beneath the cork to exchange oxygen and carbon dioxide with the air.

do thrive, however, in boggy, swampy locations. In their case, special modifications enable them to survive. Usually the roots grow very close to the surface of the soil and thus closer to the oxygen which dissolves in the water from the air. Is it surprising, then, that hurricanes often blow down more trees in low, wet locations than in higher, more exposed locations?

Another adaptation to life in swampy places is the presence of loosely packed parenchyma cells within the root and stem. Air spaces between these cells (the tissue is called the cortex) are interconnected with one another. This system of air spaces enables oxygen to diffuse internally from the stem (above water) to the roots. While this is not a very efficient system, it is, at least, adequate to supply the moderate gas-exchange needs of roots.

Gas exchange in woody stems occurs through the lenticels

Woody perennial stems likewise have only moderate needs for gas exchange. Only the cells of the bark region (epidermis, cortex, phloem, cambium, and young xylem) are alive and thus in need of oxygen for respiration. The outer cells of the bark are dead cork cells which are impregnated with a waxy, waterproof (and airproof) substance called suberin. As is the case in mature roots, though, lenticels are present, which enable oxygen to reach the living cells of the stem and carbon dioxide to be released to the atmosphere (Fig. 12.5).

In many annual plants, the stems are green and almost as important in carrying out photosynthesis as the leaves. In these stems, the gas-exchange mechanism is quite similar to that of the leaves.

12.4 GAS EXCHANGE IN THE LEAF

In the last chapter, we studied how the structure of the leaf adapts it for carrying out its main function, photosynthesis. A leaf photosynthesizing rapidly needs a substantial and steady supply of carbon dioxide. It also

Stomata regulate the movement of gases in and out of the leaf

must release an equivalent volume of oxygen during the process. The exchange of gases occurs through pores in the leaf surface, the **stomata** (singular, stoma). When the leaf is actively photosynthesizing, the carbon dioxide content of the air in the air spaces of the spongy layer falls below the 0.03% present in the outside air. As a result of the difference in concentration, carbon dioxide then diffuses from the outside air through the stomata into the air spaces of the spongy layer. Here it dissolves in the film of moisture that covers the surface of every cell in the interior portion of the leaf. Once dissolved, most of it forms bicarbonate ions:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-.$$

These enter the cells of the spongy and palisade layers by diffusion, where they are incorporated into the dark reactions of photosynthesis. Oxygen produced by the photosynthesizing cells diffuses from the cells into their surface film of moisture. From here it diffuses into the air in the air spaces and ultimately to the outside by means of the stomata.

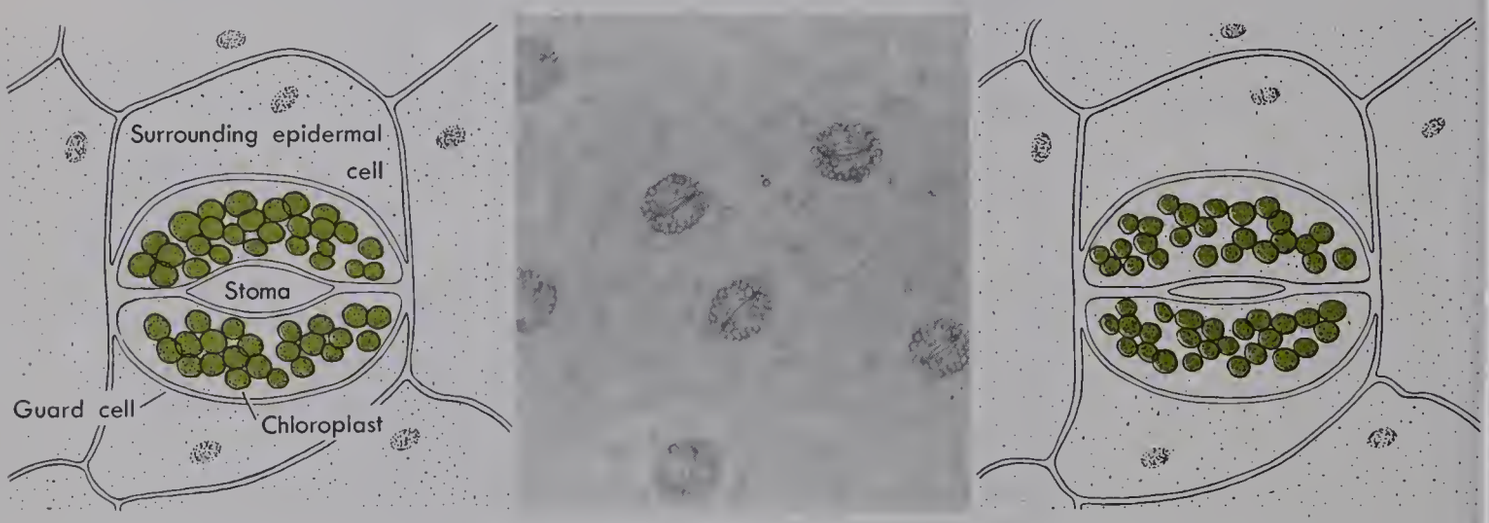


Fig. 12.6 Stomata: open (left) and closed (right). Stomata open when turgor builds up within the guard cells. Light striking the leaf initiates the reactions leading to this effect.

Mechanism of opening and closing of stomata

Under most circumstances, the stomata open when light strikes the leaf in the morning and close during the night. A great deal of experimentation has been carried out in an effort to discover how light actually controls the opening of the stomata. The immediate cause seems to be a change of turgor in the **guard cells.** The inner wall of each guard cell is thick and elastic. The outer wall is much thinner. When turgor develops within the two guard cells flanking a stoma, the thin outer walls bulge out and force the inner walls into a crescent shape (Fig. 12.6). This opens the stoma. When the guard cells lose turgor, which can be accomplished experimentally by bathing them in a solution isotonic to their cytoplasm, the elastic inner walls restore the original shape and the stoma closes.

Turgor changes

As you learned in Chapter 6, the buildup of turgor in a cell depends upon the development of a difference in osmotic pressure between that cell and its surroundings. Figure 12.7 shows the osmotic pressure at different times of day measured within the guard cells of one plant. The osmotic pressure within the other cells of the lower epidermis remained steady at 150 lb/in^2. When the osmotic pressure of the guard cells became substantially greater than that of the surrounding lower epidermal cells, the stomata opened. In the evening, when the osmotic pressure of the guard cells dropped close to that of the surrounding cells, the stomata closed.

Osmotic pressure in typical guard cells

Time	Osmotic pressure (lbs/in^2)
7 a.m.	212
11 a.m.	456
5 p.m.	272
12 midnight	191

(The osmotic pressure of the other lower epidermal cells remained constant at 150 lbs/in^2 during this experiment.)

Fig. 12.7

In order for the osmotic pressure within the guard cells to increase, their water concentration must decrease with respect to that in the surrounding cells. Put another way, there must be an accumulation of molecules and/or ions in the cytoplasm. This seems to be the case. During the day, glucose molecules and potassium ions accumulate within the guard cells and exert a strong osmotic effect.

The effect of pH on stomata

When leaves are placed in solutions with a pH lower than 6.3, the stomata close. When they are placed in solutions of a higher pH (pH 8 seems to be optimum), the stomata open. What could cause a raising of the pH during daylight hours and a falling of pH during the night? Remember that when carbon dioxide dissolves in water, carbonic *acid* is formed. This acid lowers the pH, of course. However, as mentioned above, the carbon dioxide content of the air in an actively photosynthesizing leaf decreases. This, in turn, raises the pH. One bit of evidence to support the idea that it is the amount of carbon dioxide present that controls pH (and thus stomatal opening) is that the stomata of leaves in the dark will open if exposed to air containing less than 0.03% carbon dioxide. Thus it seems that light stimulates the opening of the stomata by (1) stimulating photosynthesis in the leaf, which (2) causes a reduction in the carbon dioxide concentration of the air in the air spaces, which (3) increases the pH of the cytoplasm of the guard cells, which (4) promotes the accumulation of solutes, which (5) causes water to enter the guard cells by osmosis from the surrounding epidermal cells, which (6) causes a build-up of turgor, and the stomata open!

The actual gas-exchange structures of the leaf are primarily the moist cells of the spongy layer. As suggested above, one of the great problems of gas exchange in terrestrial organisms is keeping the cells of the gas-exchange organ or tissue moist in opposition to the drying effect of the air. The solution in the green leaf is typical of the solution in all truly terrestrial organisms. The cells involved in gas exchange are housed in a cavity within the organism. Air is brought to the moist cells by means of openings in the waterproof and otherwise gas-proof exterior. The epidermal cells of the leaf are covered with cutin. This material greatly cuts down water loss from the leaf, but also prevents any appreciable gas exchange from occurring. The stomata, however, allow for the controlled passage of gases to and from the interior of the leaf.

Any air that is not fully saturated with water vapor (100% relative humidity) will tend to dry the surfaces of the cells with which it comes in contact. Therefore, the photosynthesizing leaf, despite its protective modifications, will still lose substantial quantities of water to the air by evaporation. This water vapor then passes out of the leaf (through the stomata) in the process called **transpiration.** The transpired water must be replaced by transport of additional water from the soil to the leaves by way of the roots and stem.

Transpiration

The necessity of keeping the cells of the gas-exchange organ continually supplied with moisture may have some benefits to the plant. Transpiration (like sweating) is a cooling process and thus reduces the rate of photorespiration (see below). As we shall see in Chapter 14, transpiration also drives the transport mechanism by which inorganic nutrients are brought from the soil to the leaves.

When the surrounding air is very dry, the rate of transpiration may exceed the capacity of the root system to supply replacement water. This potentially hazardous situation is usually avoided by the closing of the stomata. Each stoma acts as a humidity sensor and closes when it is exposed to a steep humidity gradient between the interior and exterior of the leaf.

Stomata protect the plant from excessive transpiration

Even when humidity and soil moisture are high, most plants close their stomata during the middle part of hot summer days. The reason is that the rate of cellular respiration rises more rapidly with increasing temperature than does the rate of photosynthesis. Soon a point is reached when the plant is respiring more rapidly than it is photosynthesizing and thus the CO_2 concentration begins to rise. This, of course, closes the stomata just as it does after sundown when photosynthesis ceases.

The elevated daytime respiration of plants occurs not in the mitochondria but in peroxisomes (see Section 5.13), and results in the oxidation —without accompanying ATP production—of up to 30% of the freshly photosynthesized products. The process is called **photorespiration** because its rate is dependent on the rate of photosynthesis as well as temperature. It is hard to see how it can be anything but a liability to the plant. Interestingly enough, some warm-climate plants (e.g. sugar cane and corn)

Photorespiration

have biochemical and structural modifications by which they circumvent photorespiration. Not surprisingly, their productivity (Section 10.2) is very high. It is perhaps also not surprising that crabgrass is another member of this group!

GAS EXCHANGE IN TERRESTRIAL ANIMALS

The solution of most terrestrial animals to the problem of gas exchange with the air has been solved in a manner analogous to that used by the green plants. The gas-exchange organ is located within the body cavity. Here it is protected from mechanical damage and, even more important, from the excessive drying effect of the air. Frequently, the gas-exchange organ is liberally supplied with glands which replace the water unavoidably lost by evaporation.

Tracheae and lungs

Among the insects and certain other terrestrial invertebrates, gases are exchanged between the tissues and the air by means of an elaborate network of tubes, the **tracheae.** Among the air-breathing vertebrates, **lungs** are used as the gas-exchange structures.



Fig. 12.8 Left: tracheal system of the grasshopper. Right: a photomicrograph of tracheal tubes. (Courtesy of General Biological Supply House, Inc.)

12.5 TRACHEAL BREATHING

Spiracles

The tracheal system of the grasshopper is fairly typical of that found in all the vast numbers of kinds of insects (Fig. 12.8). The **tracheae** open to the outside through small holes in the exoskeleton called **spiracles.** The first and third segments in the thorax have two spiracles, one on each side. Another eight pairs of spiracles are found arranged in a line on each side of the abdomen. The spiracles are guarded by bristles which aid in filtering dust and other foreign matter from the air before it enters the tracheae. The spiracles are also guarded by valves. These are controlled by muscles and enable the grasshopper to regulate the opening and closing of the spiracles.

The spiracles lead to major tracheal tubes which lead, in turn, to ever-finer branches. These branches penetrate to every portion of the insect's body. At their extreme ends, they may be only one micron in diameter. At this end they are filled with liquid. Oxygen, diffusing through the system, dissolves in this fluid and then diffuses into nearby cells. Perhaps every cell in the insect's body is adjacent to at least one of these tiny tubes.

The construction of tracheae is quite interesting. Their walls must be fairly rigid in order not to be forced together by the weight of the surrounding tissues. This rigidity is imparted by spiraling strands of chitin in the walls of the trachea (Fig. 12.8). Nevertheless, there is a limit to the pressures which this kind of construction can withstand without deforming and closing the air passageway. This may be one reason why insects are all relatively small in size. The increased weight of the tissues of an animal the size of a rabbit, for example, would crush a system of tubes filled only with air.

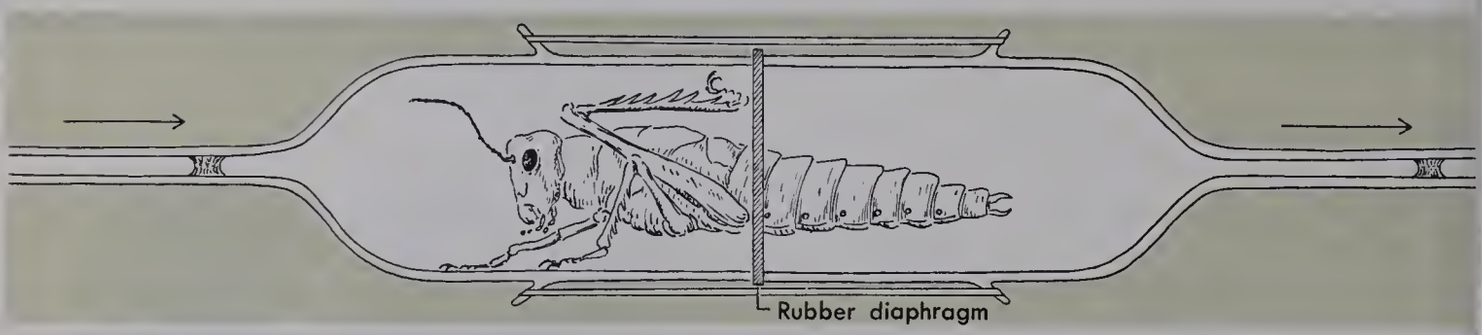


Fig. 12.9 Fraenkel's experiment demonstrating the one-way flow of air through the grasshopper. The liquid seals in the tubing move to the right as air enters the first four pairs of spiracles and is discharged through the last six pairs. The rubber diaphragm prevents air flow except through the grasshopper.

Some insects force air in and out of their tracheal system

Among the smaller or less active insects, the passage of oxygen through the tracheal system is by simple diffusion. On the other hand, large or active insects like grasshoppers forcibly ventilate their tracheae. Contraction of muscles in the abdomen compresses the internal organs and forces air out of the tracheae. As the muscles relax, the abdomen springs back to its normal volume and air is drawn into the system. Large air sacs attached to portions of the main tracheal tubes increase the effectiveness of this bellowslike action. By means of an experiment such as the one illustrated in Fig. 12.9 (first performed by the insect physiologist Gottfried Fraenkel), one can show that this action is coordinated so that a one-way flow of air is set up. Air enters the first four pairs of spiracles at the anterior end of the system and is discharged through the last six pairs in the abdomen. This one-way flow increases the efficiency of gas exchange, as oxygen-deficient air can be expelled without mingling with the incoming flow of fresh air.

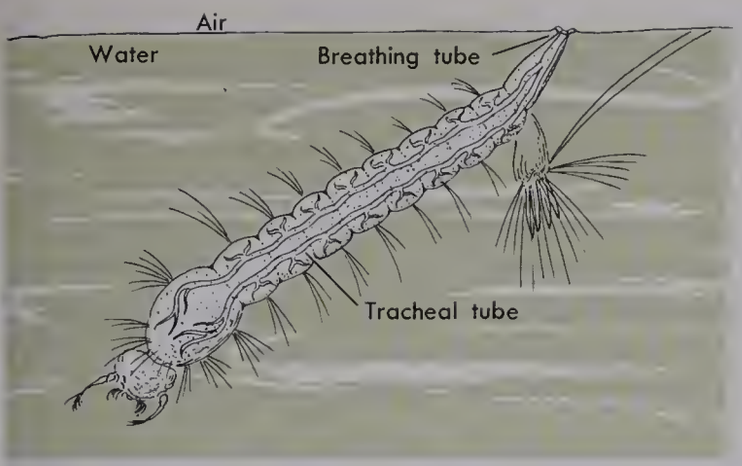


Fig. 12.10 The mosquito "wiggler" breathes air even though it lives and feeds under water.

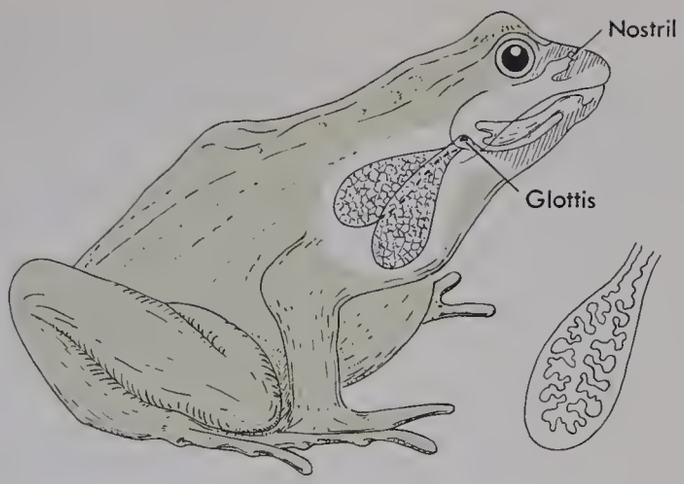


Fig. 12.11 Frog lungs. They are inflated by forcing air down through the glottis. The skin and the lining of the mouth are also used in gas exchange.

Even aquatic insects breathe by means of tracheae

Even the aquatic insects possess a tracheal system. Some of them (e.g. the mosquito "wigglers") secure their air by poking a breathing tube through the surface of the water (Fig. 12.10). This tube is connected with the tracheal system. Other insects can submerge for long periods, but in so doing, they carry with them a bubble of air from which they can breathe oxygen. Still others possess spiracles mounted on the tips of spines. With these they pierce the leaves of underwater plants and obtain oxygen from bubbles formed within the leaves. Even in those aquatic insects that have gills, after the oxygen diffuses from the water into these outgrowths, it then diffuses into a gas-filled tracheal system for transport to the tissues of the body.

12.6 LUNG BREATHERS

Among the terrestrial vertebrates (amphibians, reptiles, birds, and mammals), the organs of gas exchange are two **lungs.** In the **frog,** the lungs are two thin-walled sacs suspended in the body cavity and connected to the mouth cavity through an opening, the glottis (Fig. 12.11). The surface area of the lungs is increased by a system of inner partitions which are richly supplied with blood vessels. To inflate the lungs, the frog must first fill its mouth with air by opening its two nostrils and lowering the floor of its mouth. (This, in itself, may at times satisfy the frog's oxygen needs. The lining of the mouth is also well supplied with blood vessels and serves as a gas-exchange organ.) Then the frog closes the internal openings to its nostrils, opens its glottis, and raises the floor of its mouth. These actions force air from the mouth into the lungs, inflating them. In fact, the frog may force so much air into the lungs that they expand throughout the entire body cavity.

Oxygen in this air dissolves in the film of moisture on the surface of the epithelial cells of the lungs. The oxygen then diffuses through the

epithelial cells and into tiny blood vessels, the **capillaries.** Once in the blood, most of the oxygen enters the myriads of oval-shaped red blood cells. These floating cells contain the red pigment **hemoglobin.** Under the conditions that exist in the lungs, the hemoglobin combines chemically with the oxygen. The oxygen is then distributed throughout the frog's body in the circulating blood. When the blood reaches the various internal organs and tissues, the hemoglobin releases its oxygen. The oxygen is then free to leave the blood and enter the cells of the body to supply the needs of respiration. Carbon dioxide produced by cellular respiration enters the blood at the tissues and is then carried to the lungs and skin for release to the atmosphere.

Oxygen transport

The **skin** serves as a supplementary organ of gas exchange in most amphibians. It is richly supplied with blood vessels, and permeable to both water and gases. It can, however, function in gas exchange only if it is kept moist. Mucus-secreting cells in the skin assist in this function, but they are not adequate to cope with the drying effects of hot air of low humidity. Thus most amphibians are confined to moist locations such as ponds, swamps, damp soil, etc. This is just one of the reasons why they cannot be considered truly terrestrial animals.

In the frog, gas exchange occurs through the skin and the lining of the mouth, as well as in the lungs

Reptiles possess a scaly, dry skin. It is rather impervious to the passage of water so that little moisture is lost from the animal by this route. Thus reptiles are not confined to damp locations, although many do, in fact, live in such places. Lizards and snakes are abundant in the deserts, one of the driest habitats. While a scaly skin is an adaptation permitting safe exposure to dry air, it is of no use whatsoever as a gas-exchange organ. Reptiles depend upon their lungs for this function. Not only do their lungs have a relatively greater surface area than those of amphibians, but the ventilation of the lungs is considerably more efficient. The lungs are surrounded by a cage of rib bones (Fig. 12.12). These may alternately be spread apart and then drawn together by opposing sets of rib muscles. When they are spread apart, the volume of the rib cage increases. This expansion creates a partial vacuum within the lungs which is relieved by an inrush of fresh air. The fresh air, of course, brings a fresh supply of oxygen to the moist tissues of the lungs. Contraction of the rib cage then forces air out of the lungs. This exhaled air is depleted of oxygen but contains the carbon dioxide given off at the lungs. Some snakes exhale with great force when disturbed. The resulting hissing noise may frighten away an intruder.

Lungs are the only organs of gas exchange in reptiles

Expansion of their rib cage enables reptiles to inflate their lungs

Although the ventilation of **bird** lungs is similar in principle to that of reptile lungs, the vigor of the action is intensified during flight by the beating of the wings. Unlike reptiles, birds are homeothermic ("warm-blooded"). They maintain a constant body temperature (usually around 104°F) despite wide fluctuations in the temperature of the external environment. Birds maintain their body temperature chiefly through the heat produced by muscular activity. Muscular activity, in turn, depends upon the energy liberated by cellular respiration. It is no wonder, then, that the oxygen demands of a small, active bird are very great. Outgrowths of the

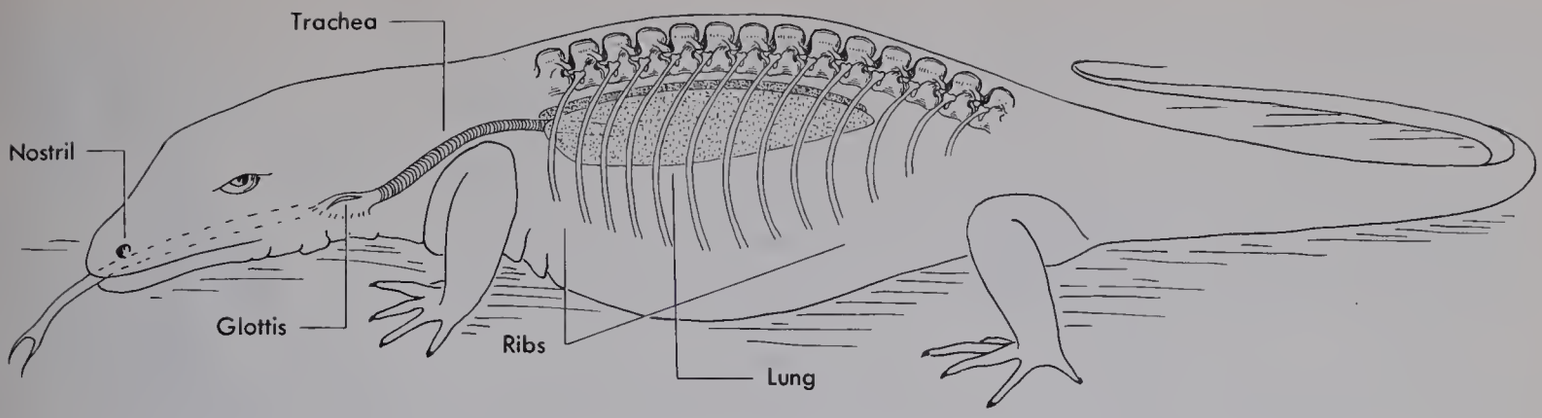


Fig. 12.12 Lizard lungs. They are inflated by expanding the rib cage.

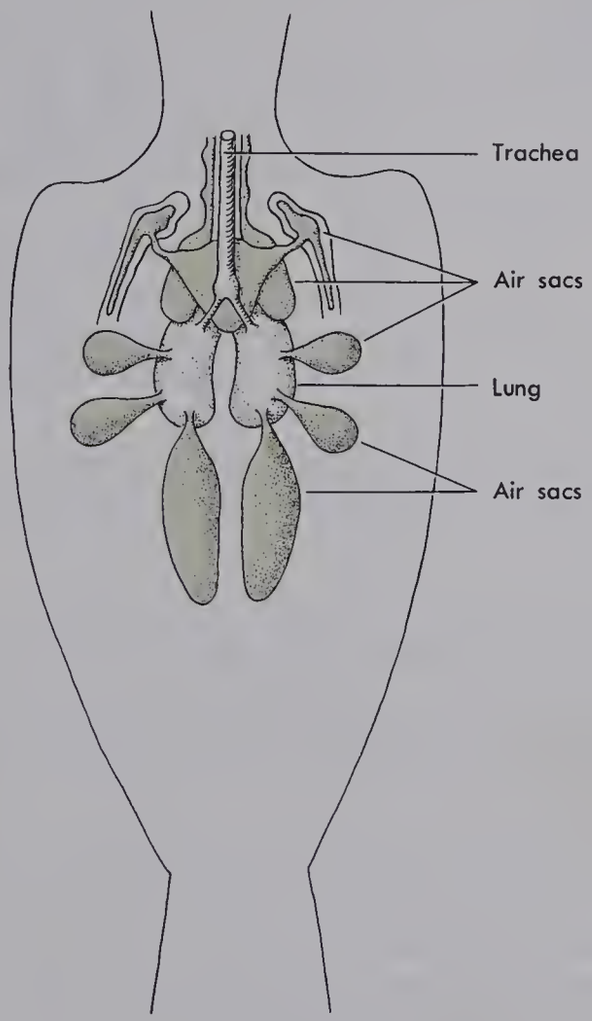


Fig. 12.13 Air sacs in the pigeon. Although no gas exchange goes on in the air sacs, they are arranged so that fresh air passes through the lungs during exhalation as well as inhalation.

lungs, the **air sacs** (Fig. 12.13), help birds satisfy these extraordinary demands. Although no gas exchange actually occurs in them, their anatomical arrangement increases the efficiency of lung ventilation by enabling fresh air to pass through the lungs during *both* inhalation *and* exhalation. The air sacs also aid in reducing the density of the body by substituting air for tissue or fluid in many locations. For example, some of the bird's bones are hollow and penetrated by air sacs.

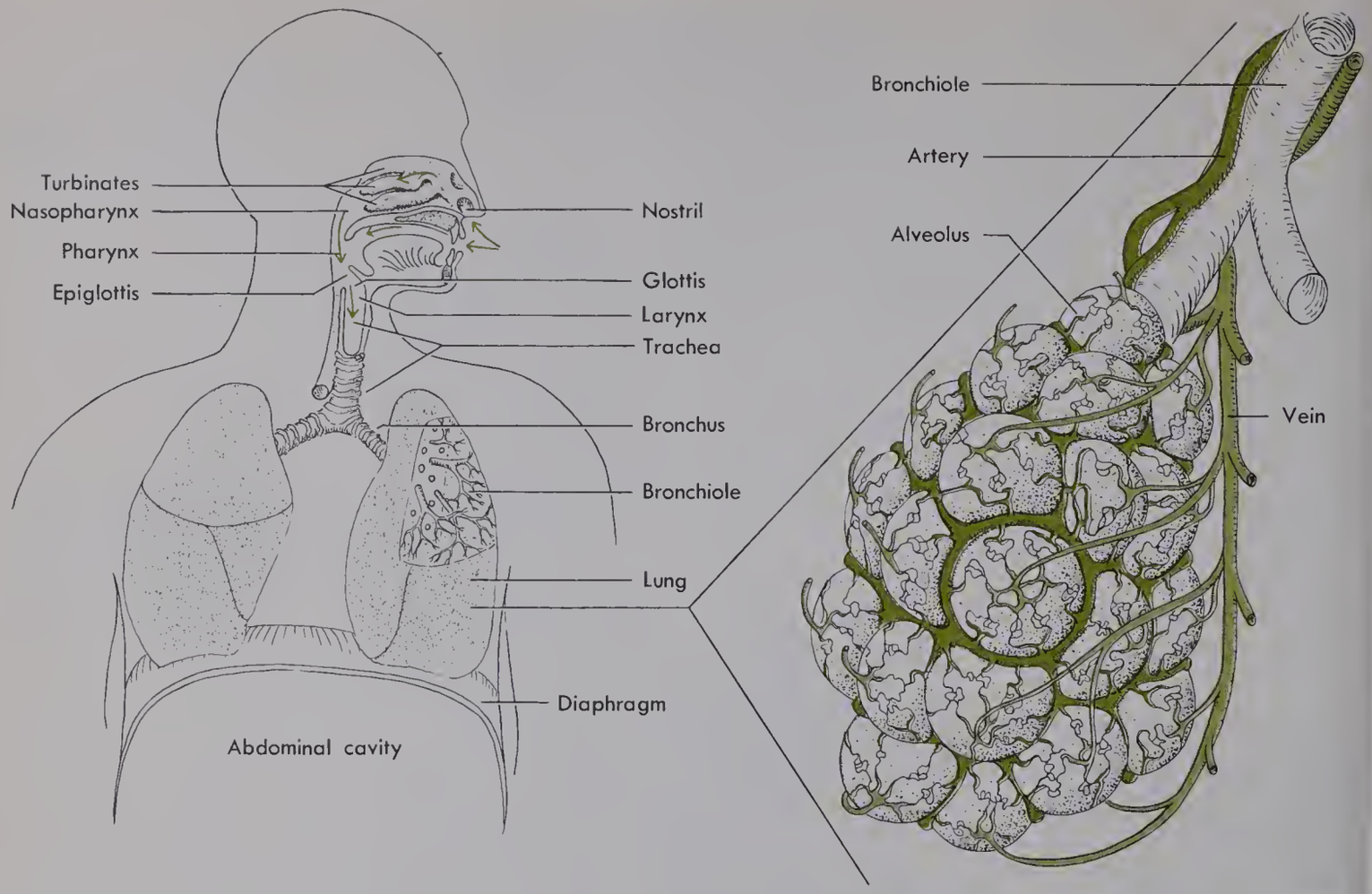


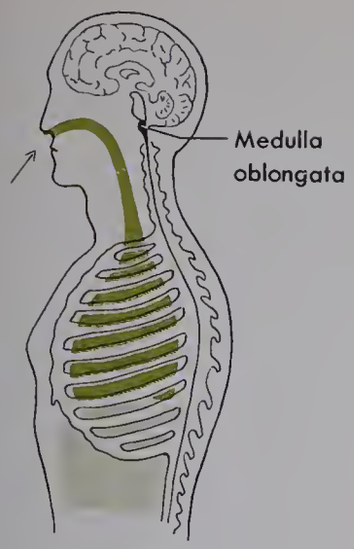
Fig. 12.14 Gas-exchange system in man. Transfer of oxygen and carbon dioxide between air and blood occurs at the alveoli.

12.7 MECHANISM OF BREATHING IN MAN

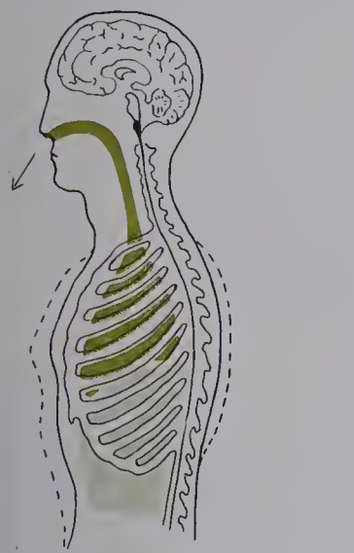
The diaphragm separates the thoracic and abdominal cavities

Among the mammals, the efficiency of lung ventilation is improved by the presence of a **diaphragm.** This is a dome-shaped, muscular partition that divides the body cavity into two compartments: the abdominal cavity (which contains the viscera—the stomach, intestines, etc.) and the thoracic cavity (which contains the heart and lungs) (Fig. 12.14). The inner surface of the thoracic cavity and the outer surface of the lungs are lined with thin membranes, the **pleural** membranes. With the aid of a film of moisture between them, the lung pleura adhere tightly to the cavity pleura.

The necessity for adhesion between the lungs and the walls of the thoracic cavity is vividly illustrated when air is introduced between the pleural membranes. The force of adhesion is broken and the natural elasticity of the lung causes it to collapse. This procedure is often done deliberately when a lung has been damaged by a disease such as tuberculosis. Collapsing the lung allows it to rest and thus heal more rapidly. Reinflation of the lung is no problem because the air between the pleural membranes is gradually absorbed by the tissues and the lung assumes its normal size once more.



INHALING



EXHALING

Fig. 12.15 During inspiration (inhaling), the ribs are lifted up and outward; the diaphragm is lowered. The rate and depth of breathing are controlled by nerve impulses originating in the medulla oblongata.

Any action which increases the volume of the thoracic cavity increases the volume of the lungs because of their adhesion to the walls of the cavity. Spreading of the rib cage accomplishes this as it does in the reptiles and birds, but in mammals (including man) the action is enhanced by contraction of the diaphragm. Contraction lowers the diaphragm, resulting in a corresponding increase in the volume of the thoracic cavity. This, in turn, stretches the lungs, and air rushes into them. When the diaphragm relaxes, it returns to its former position, permitting the lungs to return to their former size. As they do so, air is forced out of them.

The motion of the diaphragm in breathing accompanies, but does not substitute for, the action of the rib cage. The motion of the ribs is controlled by the external and internal intercostal muscles. During inspiration (inhaling), the external intercostal muscles contract, lifting the ribs up and outward (Fig. 12.15). This motion increases the volume of the thoracic cavity and hence draws air into the lungs. During expiration (exhaling), the external intercostal muscles relax and this, aided by the natural elasticity of the lungs, returns the thoracic cavity to its normal dimensions. The cycle of inspiration and expiration is repeated about 15 to 18 times each minute in the resting adult. About 500 ml of air is drawn in and then exhaled during each cycle.

It is, of course, possible to breathe more deeply, either consciously or as the result of exercise. Vigorous inspiration occurs simply as a result of more vigorous contraction of the diaphragm and the external intercostal muscles. Vigorous expiration depends, however, upon more than the elastic recoil of these muscles and the lungs. The *internal* intercostal muscles draw the ribs down and inward, thus reducing the volume of the thoracic cavity still further. At the same time, the muscular wall of the abdomen contracts, forcibly pushing the stomach, liver, etc., upwards against the diaphragm and reducing the volume of the thoracic cavity from that direction. With vigorous inspiration and expiration, an average adult male can flush his lungs with about 4 liters of air at each breath. This is known as the **vital capacity** of the lungs. Even at the point of maximum expiration, about 1200 ml of air ("residual air") remain in the lungs.

12.8 PATHWAY OF AIR IN MAN

Ventilation of the lungs is, of course, also dependent upon an unobstructed passageway between them and the outside air. During inspiration, air enters the **nostrils,** passes through the **nasal cavities** (one behind each nostril), through the **nasopharynx** and (merging with any air taken in through the mouth) the oral pharynx (Fig. 12.14). From the oral pharynx, the air passes through the **glottis** into the voice box, or **larynx.** From here it passes into the major air tube, the **trachea.** The walls of the trachea are stiffened by horseshoe-shaped bands of cartilage. These bands prevent the trachea from collapsing as a result of compression under the weight of surrounding tissues. The trachea branches into a right and left **bronchus,** leading to the right and left lungs, respectively. The bronchi, in turn,

branch into many **bronchioles.** The walls of the bronchi and the major bronchioles are also stiffened with cartilage. Each bronchiole terminates in a grapelike cluster of tiny sacs, the **alveoli.** It is only in the alveoli that gas exchange actually takes place. It has been estimated that the total surface area for gas exchange provided by the 300 million alveoli in two adult lungs ranges from 400 to 800 ft^2. Compare this remarkable value with the total skin surface area of a 150-lb, 5'10" human: 20 ft^2.

The lungs provide our largest interface between the internal and external environments

The large area provided by the alveoli is sufficient to take care of the gas-exchange needs of the body both at rest and during exertion. Under certain conditions, however, the gas-exchange area of the lungs may be seriously reduced. For example, virus or bacterial infections of the alveoli result in **pneumonia.** In pneumonia, lymph and mucus accumulate in the alveoli and bronchioles, reducing the area exposed to air. In critical cases, the patient may turn blue from oxygen starvation. Allowing the patient to breath pure oxygen under an oxygen tent may save his life. The increased concentration of oxygen inhaled makes possible the most effective use of the gas-exchange area still available.

Pneumonia temporarily reduces the surface available for gas exchange

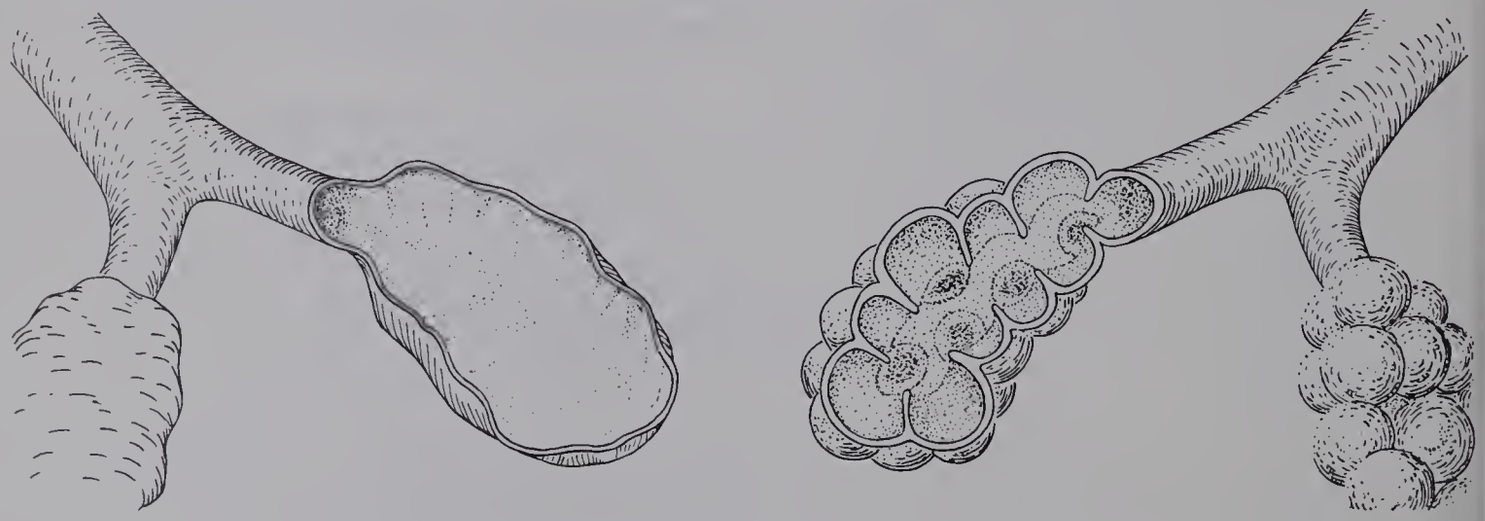


Fig. 12.16 Structure of the alveoli in a normal lung (right) and the lung of a victim of emphysema (left). Breakdown of the alveolar walls reduces the surface area for gas exchange.

Another disease of the lungs in which the gas-exchange area is reduced is **emphysema.** In this disease, many of the walls separating the alveoli become irritated and break down. This greatly reduces the surface area available for gas exchange (Fig. 12.16). Unlike pneumonia, this condition develops very slowly and is seldom a direct cause of death. However, the gradual loss of gas-exchange area forces the heart to pump ever larger quantities of blood to the lungs in order to satisfy the gas-exchange needs of the body. The added strain this imposes on the heart often leads to heart failure.

In emphysema, the surface available for gas exchange is permanently reduced

During its passage from the outside to the alveoli, inhaled air is "conditioned." In the nasal cavities, the incoming air is warmed, moistened, and filtered. These operations are hastened by the **turbinates** (Fig. 12.14),

which provide additional surface for contact between the air and the moist membranes of the nasal cavities. Air that is drawn in through the mouth is not as well conditioned as the air that enters through the nostrils.

Air from either source passes through the oral pharynx, from which leads not only the glottis but also the opening to the esophagus (Fig. 12.14). A flap of tissue above the glottis, the epiglottis, seems to aid in avoiding a conflict in function. When food is swallowed, the epiglottis automatically projects over the glottis, thus insuring that no food gets into the larynx and trachea. On the rare occasions when food or water does go down the "wrong way," a violent spasm of coughing usually expels the material.

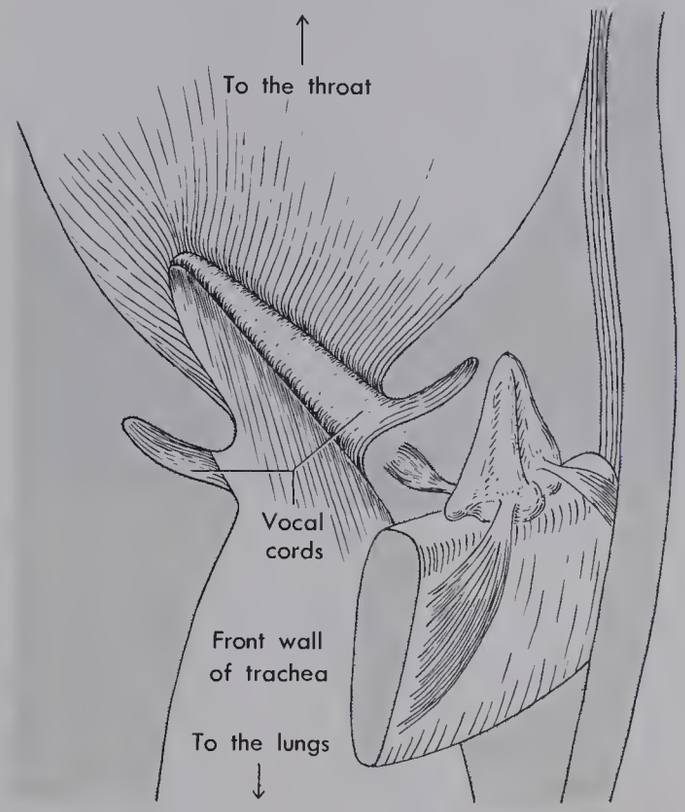


Fig. 12.17 The vocal cords. The rear wall of the larynx has been cut away. The pitch of the voice is altered by changing the tension on the vocal cords.

The larynx

The **larynx,** or voice box, is an enlarged portion of the trachea. Like the rest of the trachea, it is protected by cartilage. Associated with the deepening of the voice, which occurs during adolescence in boys, is an enlargement of the larynx. As a result of this enlargement, the cartilage covering protrudes at the front of the neck, forming the "Adam's apple."

Stretched across the larynx are two folds of tissue, the **vocal cords** (Fig. 12.17). These can be placed under varying degrees of tension through a system of muscles and cartilaginous levers. When air (usually as it is exhaled) passes over the tensed vocal cords, it causes them to vibrate. These vibrations, in turn, set the air in the larynx, pharynx, and mouth vibrating in sympathy. The air vibrations produce the sounds we use in speech and singing. Changing the tension on the vocal cords changes the pitch of the sound. Use of the tongue and other mouth parts breaks the sounds up into the various phonetic units that make up spoken language.

Occasionally the vocal cords may become infected and inflamed. Inflammation thickens them so that they do not vibrate cleanly. The result is the husky voice, or even temporary loss of voice, associated with **laryngitis.**

The inner walls of the trachea, bronchi, and bronchioles are all covered with a mucus-secreting ciliated epithelium. Fine dust particles that are not filtered out by the nose are trapped in the mucus. The cilia then sweep this material up the trachea. When it nears the glottis, the forceful exhalation of coughing is initiated, and the material, phlegm, is propelled into the mouth.

The efficiency of lung ventilation is reduced in smokers

Studies (carried out on high-school and college students) have shown that the efficiency of lung ventilation is reduced in cigarette smokers. The lungs behave as though the small bronchioles had become partially obstructed. This would occur if there were an excessive production of mucus in the lungs (and cigarette smokers do bring up more phlegm from their lungs—a condition known as chronic bronchitis) or if the rate of mucus removal by the ciliated cells were impaired. The action of the cilia *is* slowed following exposure to tobacco smoke. This has been determined by having subjects inhale an aerosol containing tiny radioactive particles. The rate at which the particles are swept out of the lungs can be followed with a large Geiger counter. It is measurably slowed in subjects who have recently been smoking, although a long-term depression has not been observed.

Tobacco smoke slows the beating of cilia

Composition of atmospheric air and expired air in a typical subject

Component	% by volume Atmospheric air	Expired air
N_2 (plus inert gases)	78.62	74.9
O_2	20.85	15.3
CO_2	0.03	3.6
H_2O	0.5	6.2
	100.0	100.0

Fig. 12.18

It is only in the alveoli that actual gas exchange goes on. A glance at the table in Fig. 12.18 shows what happens in a typical case to the composition of the air when it reaches the alveoli. Some of the oxygen dissolves in the fluid that moistens the thin epithelium of the alveoli. From here, the oxygen diffuses into the blood contained within the numerous capillaries present in the alveoli. As in the frog, most of the oxygen then combines with the hemoglobin contained within the red blood cells. Simultaneously, some of the carbon dioxide in the blood diffuses out into the alveoli, from which it can be exhaled. The circulation of the blood then carries the oxygen to all the cells of the body. In so doing, it picks up carbon dioxide from these cells for transport back to the capillaries in the alveoli. Additional details of the chemical mechanisms by which oxygen and carbon dioxide are transported efficiently by the blood will be considered in the next chapter.

Gas exchange in the alveoli



Fig. 12.19 Alveoli and an adjacent capillary from the lung of a laboratory mouse (10,500 X). Note the thinness of the epithelial cells (Ep) that line the alveolus and capillary (except where the nucleus, N, is located). The dark gray object is a red blood cell. (Reproduced with permission from Keith R. Porter and Mary A. Bonneville, *An Introduction to the Fine Structure of Cells and Tissues*, 2nd ed., Lea and Febiger, Philadelphia, 1964.)

In the alveoli, blood and air are brought within one micrometer of each other

The ease with which oxygen and carbon dioxide pass between the air of the alveoli and the red blood cells in the capillaries can be appreciated by examining Fig. 12.19. Portions of two alveoli are visible at the top, one on the left, the other on the right. Each is lined by a remarkably thin, elongated epithelial cell. The partition between the two alveoli is almost completely occupied by a capillary. Note, too, how thin is the cell that forms the wall of the capillary—except where its nucleus is located. The dark gray object within the capillary is a red blood cell. At the closest point, its surface is only 0.7μm away from the air in the alveolus, less than one-tenth of the diameter of this tiny cell!

12.9 CONTROL OF BREATHING

The rate at which cellular respiration takes place (and hence oxygen is consumed) varies with the general state of activity of the body. Vigorous

exercise may increase by 20 to 25 times the demands of the tissues for oxygen. This increased demand is met by increasing the rate and depth of breathing.

It would be reasonable to assume that lack of sufficient oxygen triggers this response. It is quite easy to show, however, that oxygen deprivation plays a very minor role in regulating the rate at which ventilation of the lungs occurs. Figure 12.20 illustrates an experimental setup by means of which a human subject can inspire various precise gas mixtures. While the subject is inhaling the gas mixture, his rate and depth of breathing can be checked constantly. The subject begins the experiment by breathing pure air (21% oxygen, 0.03% carbon dioxide, and about 79% inert gases by volume). He breathes first from the room and then from the tank. This procedure provides a "control." It reveals what, if any, changes in the subject's responses can be expected just by having him breathe from the tank. Nervousness, an unpleasant taste imparted to the air by the equipment, or increased air resistance are just three factors that might alter the subject's responses. These alterations would have to be taken into account in interpreting any changes brought about by varying the composition of the inhaled gases.

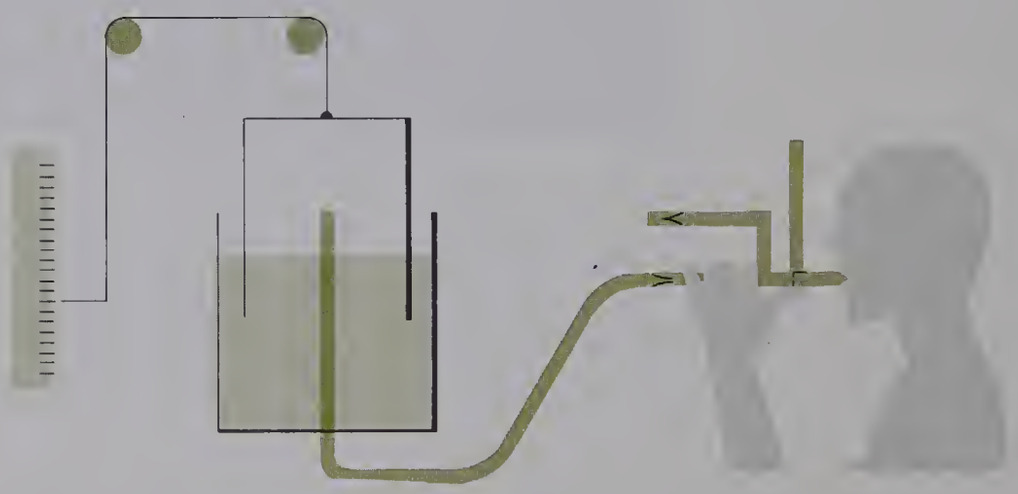


Fig. 12.20 Apparatus for determining the effects of different gas mixtures on rate and depth of breathing.

The rate and depth of breathing is controlled by the CO_2 content of the blood

The two graphs in Fig. 12.21 show that no appreciable change does, in fact, occur in this particular experiment. When 100% oxygen is substituted for air, no marked change in rate or depth of breathing occurs either, although there is a tendency for depth of breathing to decrease slightly. When the subject inhales a gas mixture consisting of 92% oxygen and 8% carbon dioxide, however, a most dramatic increase in rate and depth of inspiration takes place. Note that there is no question here of the subject's tissues suffering from a lack of oxygen. He is inhaling a gas mixture over four times as rich in oxygen as the air. The experiment suggests that the concentration of carbon dioxide plays a decided part in governing the rate and depth of breathing.

Other physiological experiments have shown that carbon dioxide achieves this effect through its action on a portion of the brain called the

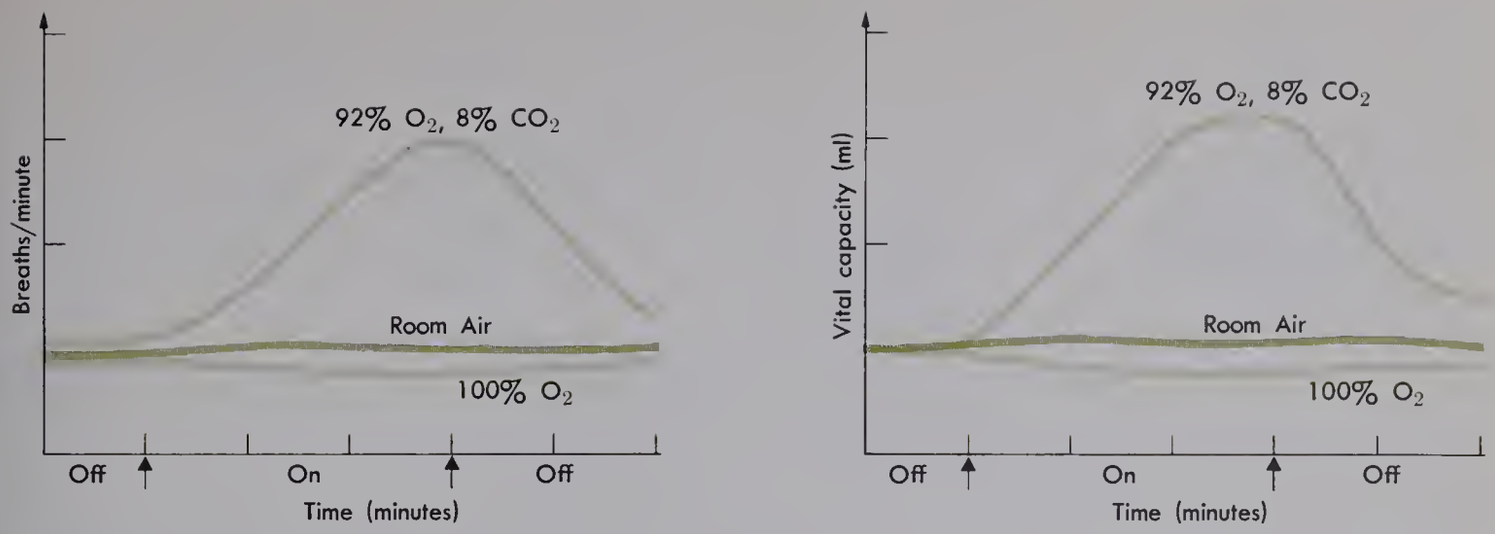


Fig. 12.21 Graphs showing effect of inspiring room air, 100% oxygen, and 92% oxygen, 8% carbon dioxide on breathing rate (left) and depth of breathing (right).

The CO_2 content of the blood is regulated by the medulla oblongata

medulla oblongata (Fig. 12.15). When blood passes through the alveoli, its carbon dioxide content becomes the same as that of the alveolar air. Moments later, the blood reaches the medulla oblongata, which contains cells that are very sensitive to the concentration of carbon dioxide in the blood. If this carbon dioxide content rises above normal levels, the medulla oblongata responds by increasing the number and rate of nerve impulses which control the action of the intercostal muscles and diaphragm. The result is an increased rate of lung ventilation, which quickly brings the carbon dioxide concentration of the alveolar air, and then of the blood, back to normal levels.

We all know that rate and depth of breathing are also under conscious control, but this conscious control has definite limits. You can hold your breath for only a limited period of time. Eventually, the carbon dioxide content of the blood reaching the medulla oblongata becomes so high that the medulla overrides conscious control. This is the "breaking point."

Experiments with the pneumograph

Figure 12.22 illustrates an experimental setup with which this type of response can be studied. Movements of the subject's chest are detected by a hollow bellows (the pneumograph) strapped around the chest. Expansion and contraction of the bellows cause decreases and increases in the pressure of the air within. These pressure changes can then be transmitted to a recording stylus, which writes on a slowly revolving, smoked drum (the kymograph). Note that after a period of breath-holding, the rate and depth of inspiration are markedly greater than before the period of breath-holding began. This can be accounted for by the build-up of carbon dioxide during the breath-holding period. The length of time that one can hold his breath to the breaking point can be increased substantially by breathing extra fast and extra deeply just prior to the period of breath-holding. Vigorous, forced ventilation reduces the carbon dioxide content of the alveolar air and blood to below its normal value, and thus it takes longer

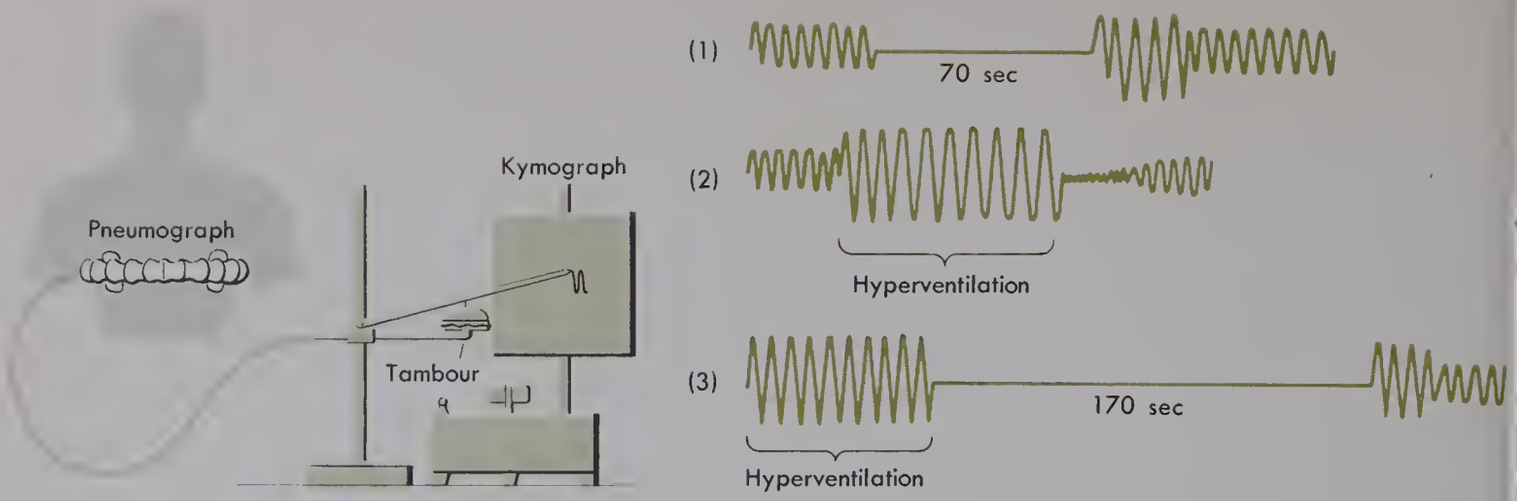


Fig. 12.22 Operation of the pneumograph and typical kymograph records showing the effect upon rate and depth of breathing of (1) breath-holding, (2) hyperventilation, and (3) hyperventilation followed by breath-holding.

for the carbon dioxide content to build up to the breaking point. If the subject simply breathes naturally after a period of extra-vigorous breathing (hyperventilation), we find that his depth of breathing is markedly less than that which preceded the period of hyperventilation.

The medulla oblongata, then, is an extremely efficient mechanism for controlling the carbon dioxide content of the blood. Any increase in carbon dioxide is quickly reduced by increased ventilation of the lungs. Any reduction in carbon dioxide content is quickly compensated for by a decreased ventilation of the lungs. The medulla is thus one of the homeostatic devices of the body. Through its activity, the carbon dioxide content of the blood is maintained within very narrow limits, and as a result, the constancy of the internal environment with respect to carbon dioxide content is maintained.

It may seem curious that the rate at which one breathes and thus supplies oxygen to the body is controlled by the carbon dioxide content of the blood rather than the oxygen content. Remember, though, that in the breakdown of glucose by cellular respiration, carbon dioxide is produced as fast as oxygen is consumed. The muscles of the body need increased amounts of oxygen during vigorous exercise, but they produce increased amounts of carbon dioxide at the same time. This triggers increased ventilation of the lungs and thus automatically provides for the greater oxygen need.

CO_2 is used in resuscitation

Knowledge of the mechanism by which breathing is controlled has led to advances in the use of resuscitators. Victims of drowning accidents, electrocution, or carbon monoxide poisoning may have their breathing stopped but not their heartbeat. Quick action in forcing air into the lungs may save their lives. In such circumstances, the tissues, especially of the heart and brain, need oxygen. Many ambulances are equipped with resuscitators containing tanks of compressed oxygen which can be forced into

the victim's lungs. It is also important that the victim's natural breathing movements be restored. To this end, it is now common practice to equip resuscitators with tanks containing a mixture of 95% oxygen (for the needs of the tissues) and 5% carbon dioxide (to stimulate the medulla oblongata).

The oxygen content of the blood exerts some control over the rate and depth of breathing

There is one situation in which man may find himself suffering from a shortage of oxygen without a corresponding increase in the production of carbon dioxide. When suddenly transported (e.g. in a nonpressurized airplane) to altitudes above 13,000 ft, he may begin to suffer oxygen deprivation. With decreased amounts of oxygen for cellular respiration there is, however, a corresponding decrease in the amount of carbon dioxide. Consequently the medulla is not stimulated to step up the rate of lung ventilation. Fortunately, the body does possess a mechanism to cope with this situation. In the aorta and carotid arteries (Fig. 13.7) there are receptors which detect reduced levels of oxygen in the blood. These receptors send impulses to the medulla oblongata and stimulate it to increase the rate of lung ventilation. Although this increase in rate and depth of breathing is not so dramatic as that brought about by carbon dioxide excess, it does bring increased quantities of oxygen to the alveoli.

12.10 AIR POLLUTION AND HEALTH

The alveolar surface provides a large and intimate interface between the internal environment and the air. Over a period of 24 hours, we breathe in some 15,000 liters of air. The purity of this air has become a matter of considerable concern.

Primary air pollutants

A number of ingredients find their way into air, chiefly as a result of man's activities, and these may fairly be considered as contaminants or air pollutants. The sources of air pollution are exceedingly varied but can be divided into two categories. One is our industrial and technological activities. Petroleum refineries, smelters (Fig. 12.23), fuel-fired electrical generating plants, and the ubiquitous automobile all release enormous quantities of pollutants into the air. These include:

1) soot (from unburned fuel) and industrial ashes of other sorts.

2) sulfur dioxide (SO_2) from the oxidation of fuels (e.g. coal, oil) containing sulfur compounds.

3) various hydrocarbons (from the incomplete combustion of gasoline). Among these is benzopyrene, a notorious carcinogen (cancer-causing agent).

4) oxides of nitrogen (e.g. NO_2) produced by the chemical union of O_2 and N_2 in the cylinders of internal combustion engines).

Secondary air pollutants

In bright sunlight, nitrogen oxides, hydrocarbons, and oxygen interact chemically to produce powerful oxidants like ozone (O_3) and peroxyacetyl nitrate (PAN). These *secondary* pollutants are exceedingly damaging to plant life and lead to the formation of smog of the type common in Los



Fig. 12.23 Copper Basin, Tennessee. The vegetation in this region was killed by deliberate burning and air pollution from a copper smelter prior to 1907. Over half a century later, continued erosion still hampers attempts at reforestation. Average annual rainfall is less and temperatures are higher than in surrounding regions. (Courtesy of the U.S. Forest Service.)



Fig. 12.24 Areas (color) of above-average mortality from cancer of the respiratory system (trachea, bronchi, and lungs) in England, Scotland, and Wales.

Angeles. PAN is primarily responsible for the eye irritation so characteristic of this type of smog.

Cigarette smoke contains benzopyrene

The other major category of air pollution is cigarette smoke. (Pipes and cigars are usually not inhaled; consequently the concentration of their smoke reaching the lungs is not nearly so high as with cigarettes.) Cigarette smoke contains a number of hydrocarbons, including benzopyrene.

Do the protective mechanisms working in the lungs provide adequate protection from air pollution? The question is not an easy one to answer. The most promising approach to an answer has come from studies in which the rate of human illness has been determined in populations differing in their exposure to air pollutants. Figure 12.24 shows that the rate of lung cancer in Great Britain is substantially higher in urban areas where there is plenty of heavy industry. Unfortunately, data of this sort are a little difficult to interpret because city dwellers are also apt to be heavier cigarette smokers than their rural cousins.

Lung cancer is more common in cities

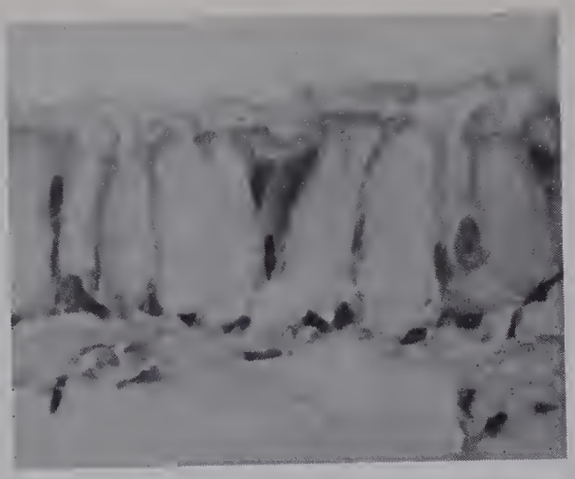
Nevertheless, as more and more data are collected, it is becoming apparent that a variety of human diseases are more common among persons exposed to the air pollution of urban life *or* to the individualized air pollution of cigarette smoking (or, especially, both). These diseases include chronic bronchitis (and its probable sequel, emphysema), asthma, several circulatory disorders, and lung cancer. In fact, if one analyzes death rates from *all causes* among United States males, the rate is directly proportional to (a) number of cigarettes smoked per day, (b) depth of inhalation, and (c) number of years since smoking began. Fortunately the processes at work—whatever they are—appear to be at least partially reversable. The overall death rate among *former* smokers is inversely proportional to the number of years elapsed since they gave up the habit.

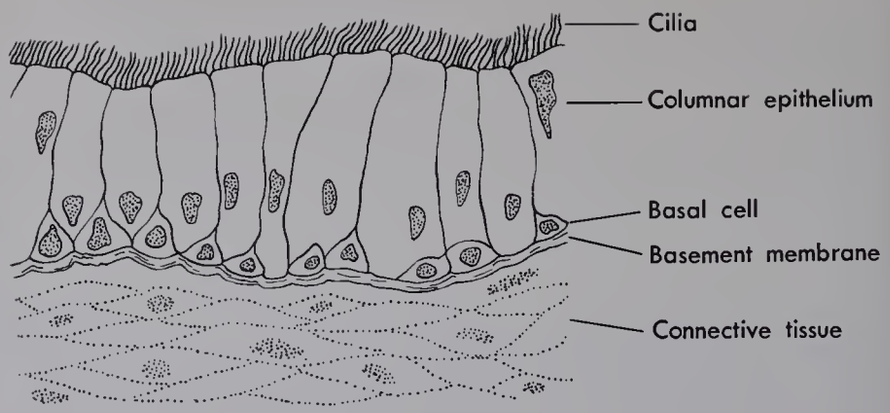
Death rates are higher among smokers

Chronic irritation of the bronchial epithelium produces a well-defined sequence of cellular changes. These include a thickening of the epithelial layer, a loss of the ciliated cells, and the appearance of cells with bizarre nuclei (Fig. 12.25). The extent of these changes has been clearly shown to be related to the amount of cigarette smoking. Cells with bizarre nuclei, which are probably **precancerous,** are quite rare in nonsmokers and their numbers do not increase with advancing age. In smokers, on the other hand, epithelial cells with bizarre nuclei are quite common and become increasingly so with increasing number of years of smoking. Fortunately, the number of such cells declines slowly once smoking is given up.

Smoking produces predictable changes in the bronchial epithelium

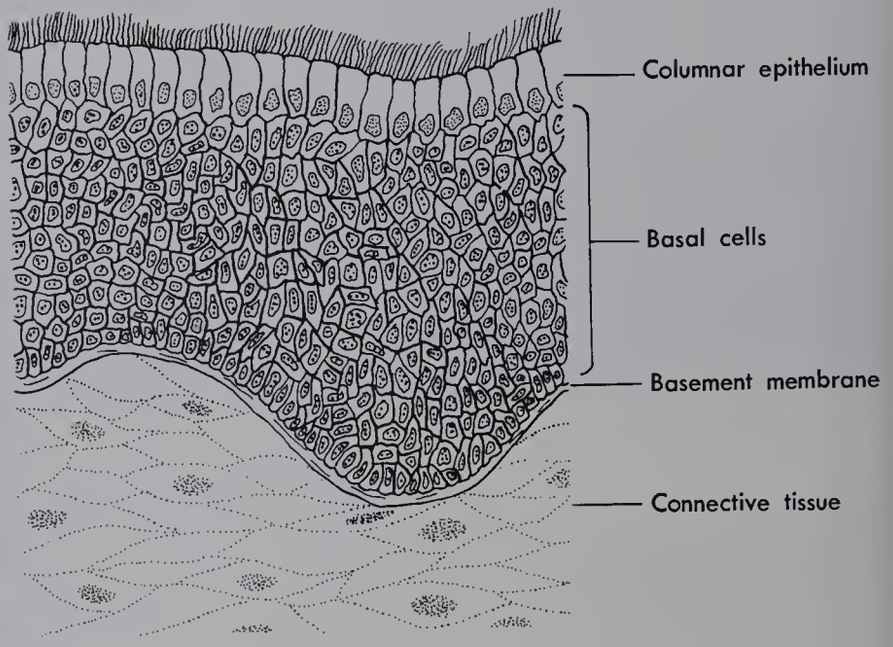
(a)



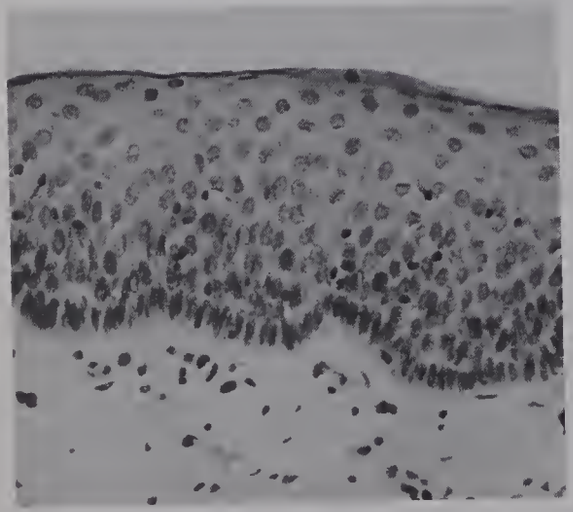

Cilia
Columnar epithelium
Basal cell
Basement membrane
Connective tissue

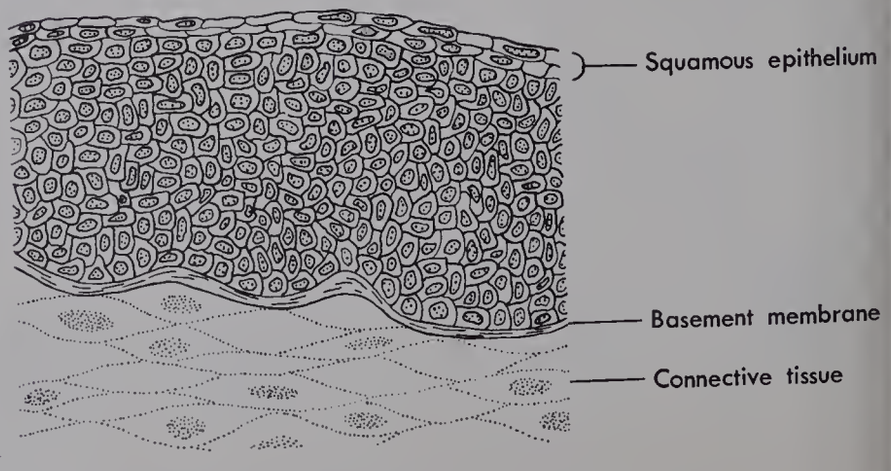
(b)




Columnar epithelium
Basal cells
Basement membrane
Connective tissue

(c)




Squamous epithelium
Basement membrane
Connective tissue

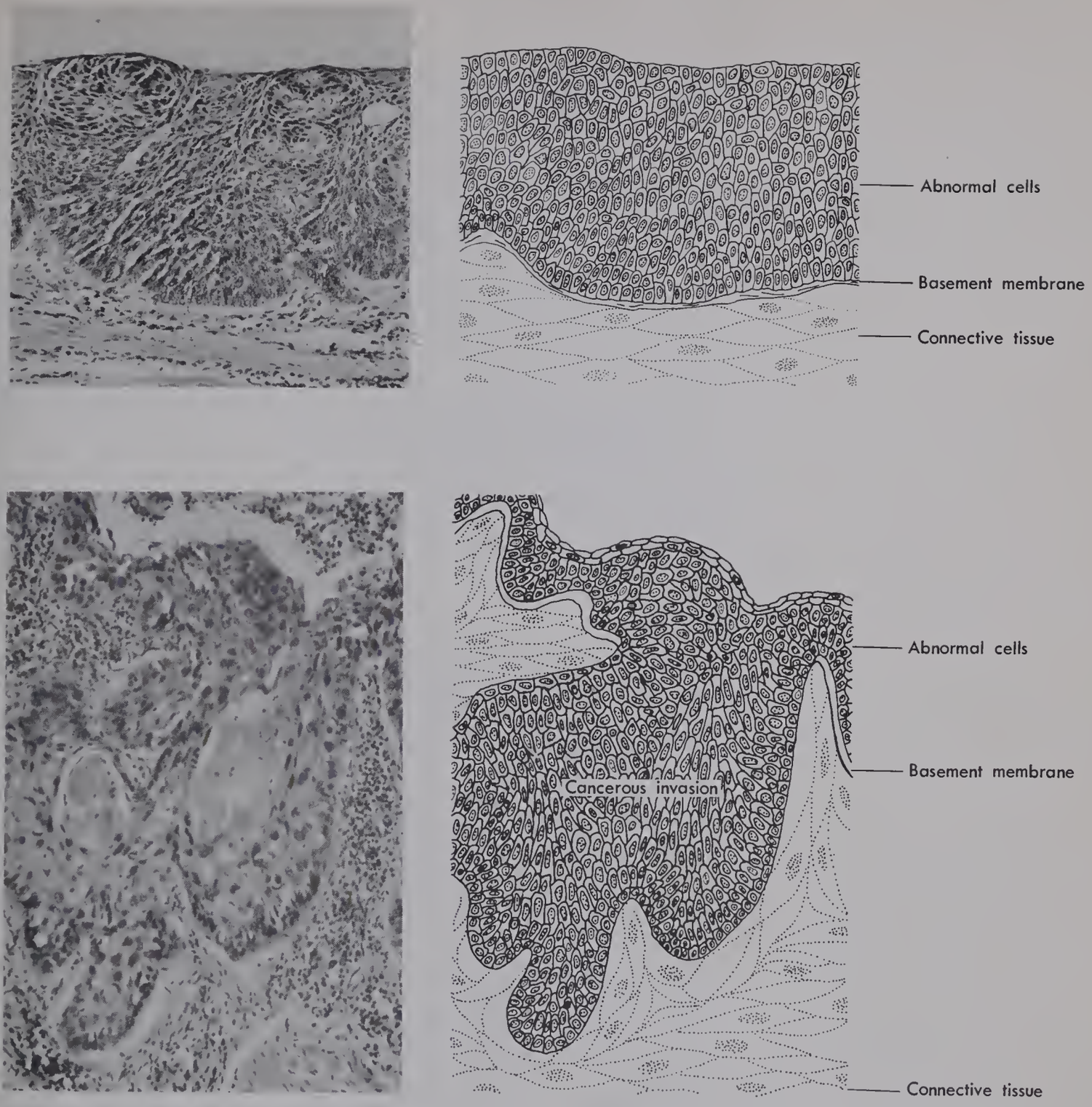


Fig. 12.25 Photomicrographs of (a) the normal epithelium of the bronchi (greatly enlarged); (b, c, d) three stages of abnormal changes to the bronchial epithelium commonly found in smokers; and (e) a lung cancer. The drawings show the cellular organization occurring at each stage. (Photomicrographs courtesy of Oscar Auerbach, M.D.)

EXERCISES AND PROBLEMS

1. Trace the pathway taken by a molecule of oxygen from the time it enters a nostril until it enters a red blood cell.
2. How do reptiles, birds, and mammals improve the efficiency of lung ventilation over that of amphibians?
3. How do amphibians compensate for their low efficiency of lung ventilation?
4. What biochemical advantage is there in the mouth-to-mouth method of artificial respiration that is not found in the older methods?
5. Using a microscope with a 43X objective and a 10X eyepiece, a student sees six stomata on the lower epidermis of a lily leaf. The diameter of the field of view is 0.4 mm. How many stomata are present per square centimeter?
6. Highway construction often interferes with the natural drainage of water, causing water to accumulate in formerly well-drained woods. Soon dead trees are to be seen in these areas. Explain.
7. Why are gills poorly adapted for life on land?
8. Which of the following gas mixtures would you use in an attempt to revive a victim of drowning: (a) 100% O_2, (b) 80% N_2, 20% O_2, (c) 95% O_2, 5% CO_2, (d) 50% O_2, 50% CO_2?

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13 TRANSPORT IN ANIMALS

Simple Transport Mechanisms

A "Closed" System: the Earthworm

An "Open" System: the Grasshopper

The Squid

Single Pump: the Fish

Three Chambers: the Frog and the Lizard

Four Chambers: Birds and Mammals

The Pathway of Circulation in Man

Blood

Functions of the Blood

The Lymphatic System

The Control of Circulation

Clotting of Blood

13.1 SIMPLE TRANSPORT MECHANISMS

Tiny organisms do not need a circulatory system

Microorganisms and tiny animals have no need for a special transport system. Diffusion, active transport, and cytoplasmic streaming are sufficient to ensure that every portion of their body is adequately supplied with materials. The food vacuoles formed in the amoeba (Fig. 7.1) are swept by streaming cytoplasm throughout the cell as digestion of the food contents occurs. The digested food molecules then pass by diffusion or by active transport into the cytoplasm. Even an animal as complex as the planarian can get along without a true circulatory system. Although there is a small amount of liquid, which bathes the internal organs of the planarian, the only motion imparted to it is that which results from the random body movements of contraction and expansion. We have seen (Section 12.1) that the shape of the planarian makes it unnecessary to have a special system for the transport of oxygen and carbon dioxide. The necessity for extensive transport of digested food materials is eliminated by a highly branched gastrovascular cavity (see Fig. 7.3). Digestion in the planarian is intracellular, and no portion of the body is far removed from the endocytic cells which line the gastrovascular cavity and accomplish this work.

13.2 A "CLOSED" SYSTEM: THE EARTHWORM

Four components of a circulatory system

The earthworm is relatively large and complex. It has a circulatory system for the transport of materials, and all of the features essential to an efficient circulatory system are found in it. These are: (1) a fluid in which the materials to be transported are dissolved, (2) a system of conducting vessels or channels in which the fluid flows, (3) a pump for maintaining the flow of the fluid, and (4) specialized organs to carry out exchanges between the fluid and the external environment. These include organs (such as the skin and intestine) that add materials to the fluid, and organs (such as the skin and excretory organs) that remove materials from the fluid and deposit them back into the external environment.

The circulating fluid of the earthworm is blood. It is mostly water in which are dissolved gases, sugars, amino acids, salts, and many other molecules and ions that play a role in the metabolism of the earthworm. The efficiency of the earthworm's blood as a medium for oxygen transport is increased by the presence of the red, oxygen-carrying pigment **hemoglobin.** The earthworm's hemoglobin is not contained within red blood cells as ours is, but is simply dissolved in the blood.

The blood of the earthworm is confined at all times to a closed system of vessels

The blood of the earthworm is transported by an elaborate system of blood vessels. It can only carry on its function of exchanging materials with individual cells, however, when it passes through the finest of the blood vessels, the **capillaries.** Because the blood is always retained within the system of blood vessels, we say that the earthworm has a "closed" system. The pump that forces the blood to the capillaries consists of five pairs of aortic loops (Fig. 13.1). Muscular contraction of the walls of these aortic loops forces blood into the ventral blood vessel. The ventral blood

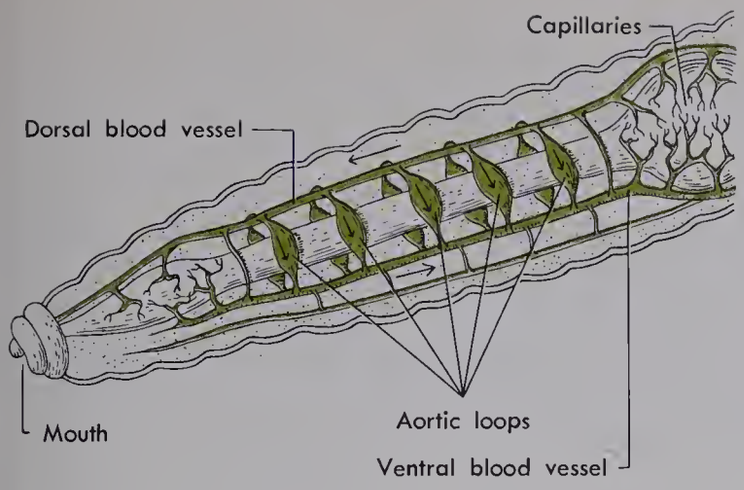


Fig. 13.1 Circulatory system of the earthworm. The blood is confined to the blood vessels at all times. Contraction of the dorsal blood vessel and the five pairs of aortic loops keeps the blood flowing.

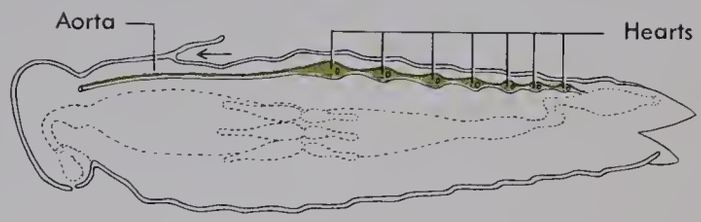


Fig. 13.2 The circulatory system of the grasshopper is "open." The blood is confined to vessels during only a small part of its circuit through the body.

vessel transports blood towards the rear of the worm and distributes the blood to an elaborate system of lesser blood vessels. All of these terminate in capillaries, and it is here that exchanges between the *exchange organs* and the *blood* and between the *blood* and the *tissues* take place. Once through the capillary beds, the blood is picked up by a second system of vessels leading into the dorsal blood vessel. This vessel contracts rhythmically, forcing the blood back to the aortic loops at the anterior end of the worm.

13.3 AN "OPEN" SYSTEM: THE GRASSHOPPER

In an open system, blood bathes the tissues directly

The circulatory system found in insects differs from that of the earthworm in one important respect. The blood is confined to vessels during only a portion of its circuit through the body. The remainder of its journey takes place within the body cavity itself. Such a system is known as an "open" circulatory system. The volume of blood required for such a system is kept to a practical level by a marked reduction in the size of the body cavity. This reduced body cavity is called a **hemocoel.** The efficiency of flow and distribution of the blood is maintained by having the hemocoel divided into chambers called sinuses.

In the grasshopper, the closed portion of the system is confined to a single long tubular heart and aorta running along the dorsal side of the insect (Fig. 13.2). The heart pumps blood into the sinuses of the hemocoel, where exchanges of materials take place. Coordinated movements of the body muscles gradually bring the blood back to the sinus surrounding the heart, the dorsal sinus. Between contractions, tiny valves in the wall of the heart open and permit the blood to enter the heart from the dorsal sinus, thus completing the circuit.

The open circulatory system of the grasshopper might seem to be quite inefficient in comparison with the closed system of the earthworm.

The blood of insects does not carry gases to and from the tissues

It should be kept in mind, however, that there is a decided difference in the function accomplished by the two systems. Remember that in the grasshopper the exchange of oxygen and carbon dioxide is accomplished by means of the tracheal system. Blood plays no part in this process. There is not even an oxygen-carrying pigment in the blood of the grasshopper. A good deal of experimentation and study has shown clearly that it is the problem of gas transport that poses the most serious demands upon a circulatory system. In animals such as the grasshopper and other insects, where the gas-exchange system is quite independent of the circulatory system, the demands upon the latter are far less severe than in animals (e.g. the earthworm) where the functions are combined.

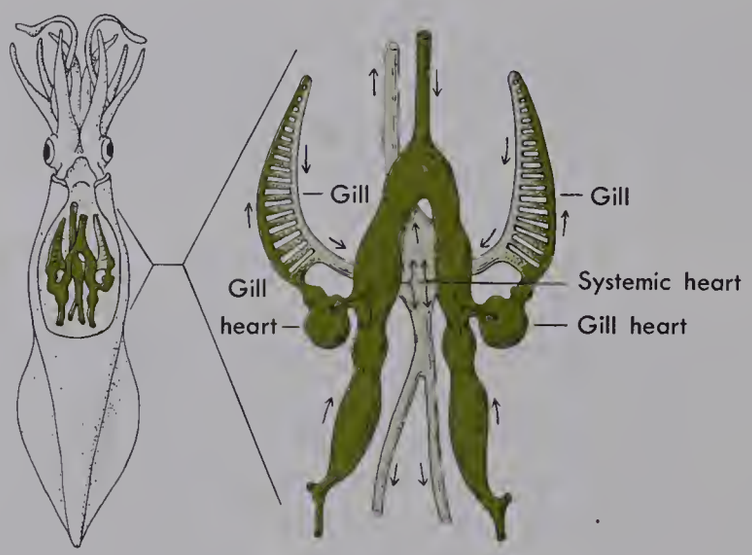


Fig. 13.3 Circulatory system of the squid. The gill hearts pump oxygen-deficient blood (dark color) to the gills where gas exchange takes place. The systemic heart pumps oxygen-rich blood (light color) to all parts of the body.

13.4 THE SQUID

The closed circulatory system of the squid is especially interesting because there are three separate pumps to maintain circulation (Fig. 13.3). One heart pumps blood to all the internal organs and tissues of the body. The other two hearts simply serve to pump blood from these internal organs and tissues to the gills, where gas exchange takes place. To understand the

The pressure created by the pumping of the heart is largely dissipated in the capillaries

advantage of such a system, it is important to realize that the pressure produced by the contraction of a heart is almost entirely dissipated when blood enters the capillaries. Although capillaries are tiny, the total cross-sectional area of all the capillaries supplied by a single major blood vessel is considerably greater than the cross-sectional area of the blood vessel itself. The situation can be compared to that which occurs when a rapidly flowing, narrowly confined stream is allowed to spread out over a flat plain. The force and velocity of flow diminish rapidly. The same situation occurs in a network of capillaries. Note, too, that gas exchange must take place in two different locations: the gills and the tissues. In both cases, the exchanges occur only while the blood is passing through capillaries. Thus, in passing through the gills, the blood loses the pressure which could then distribute it quickly to the tissues. On the other hand, in passing through

the capillaries of the tissues, the blood loses the pressure which could then force it quickly back to the gills. The squid's system of separate hearts thus copes with the problem very nicely.

It is worth noting here that a closed circulatory system with separate pumps is also found in the birds and mammals, but the two pumps are located together. One half of the bird or mammal heart pumps blood to the lungs; the other half pumps blood to the tissues. The development of separate pumps in these animals was entirely independent of that in the squid.

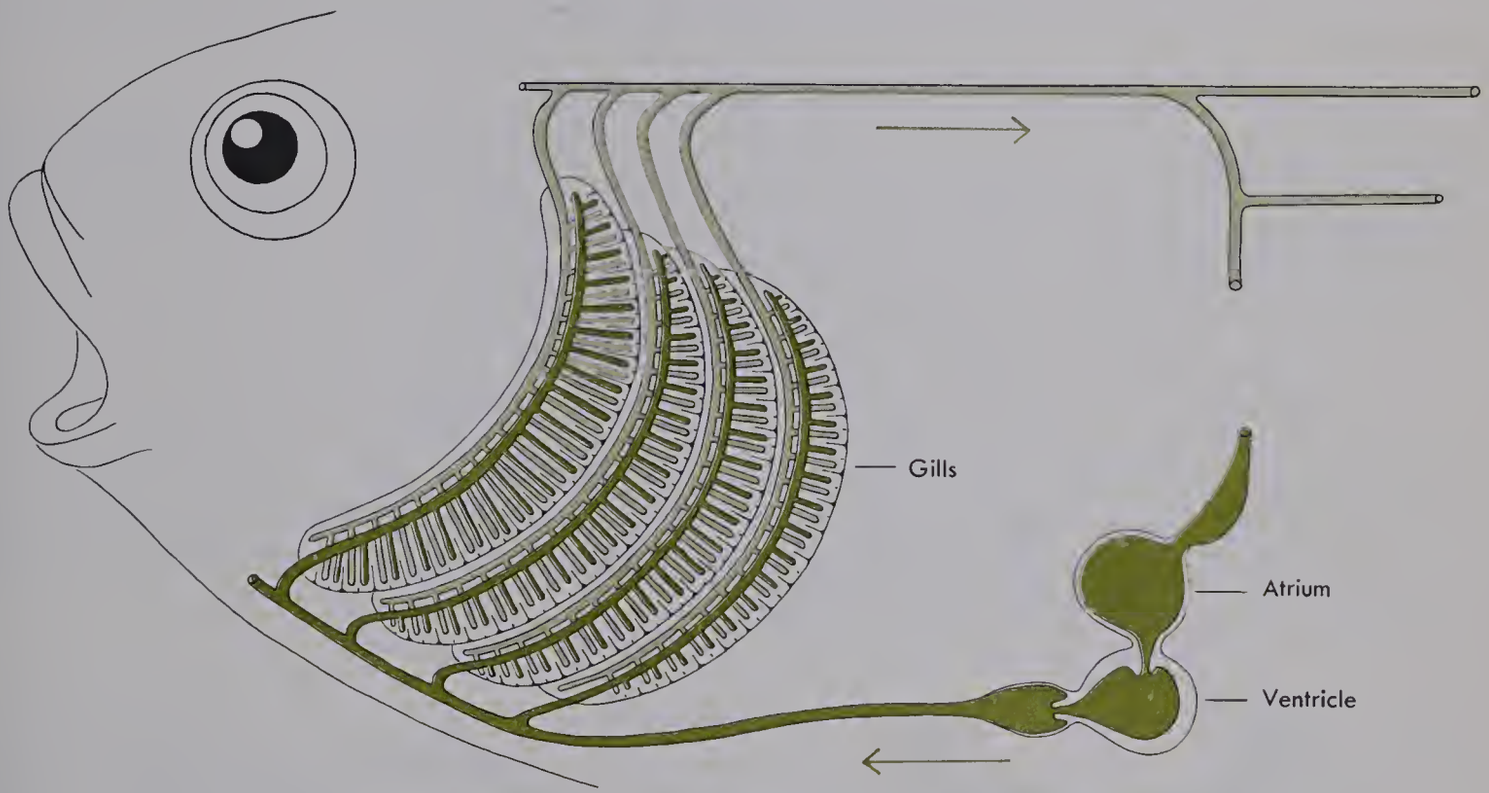


Fig. 13.4 Circulatory system of a fish. Oxygen-deficient blood (dark color) is pumped forcibly to the gills. Little pressure remains to move the oxygen-rich blood (light color) to the rest of the body.

13.5 SINGLE PUMP: THE FISH

Actually, the earliest vertebrates had only a single pump. This arrangement still persists among the modern fishes. Blood collected from throughout the fish's body enters a thin-walled receiving chamber, the **atrium.** During relaxation of the heart, the blood passes through a valve into a thick-walled, muscular **ventricle.** Forcible contraction of the ventricle forces the blood out to the capillary network of the gills. Here gas exchange takes place. From the gills, the blood passes to the many capillary networks in the remainder of the fish's body, where exchanges with the tissues take place. Then the blood returns to the heart. The system is "closed," as the blood is contained within vessels throughout its entire circuit (Fig. 13.4).

The circulatory system of fishes, while obviously adequate to their needs, is not very efficient. As mentioned above, there is a marked pressure drop when blood flows through a capillary network. In the fishes, after the blood passes through the gills, it is no longer transported vigorously by the force of the heartbeat.

13.6 THREE CHAMBERS: THE FROG AND THE LIZARD

The situation in this respect is somewhat improved in the amphibian heart. The frog heart consists of three main chambers, two atria and a ventricle. The right atrium receives oxygen-deficient blood from the blood vessels (veins) that drain the various tissues and organs of the frog's body. Blood from the lungs, rich in oxygen, is carried to a separate atrium on the left. The blood from both atria passes into a single ventricle (Fig. 13.5). Contraction of the ventricle forces the blood into a vessel which immediately divides into a left and a right branch. Each of these immediately branches again into three main arteries. The anterior arteries carry blood to the head and brain of the frog. The middle ones, the systemic arches, carry blood to the internal tissues and organs in the rest of the body. The posterior arteries carry blood to the skin and lungs.

The single ventricle of the frog heart pumps oxygen-rich and oxygen-deficient blood with surprisingly little mixing

At first glance, it might seem that whatever advantage had been gained by returning oxygen-deficient blood to one part of the heart and oxygen-rich blood to another would be lost by mixing the two bloods in the single ventricle. To some extent this is true. However, the ventricle is partially divided into narrow chambers which tend to reduce mixing of the two bloods. When the ventricle contracts, most of the oxygen-deficient blood is deflected, with surprisingly little mixing, into the two arteries leading to the skin and lungs. Here a fresh supply of oxygen will be picked up. Oxygen-rich blood from the left atrium is sent, relatively pure, into the arteries leading to the brain. Only the blood passing into the systemic arches leading to the rest of the frog's body has been thoroughly mixed, but even so it contains enough oxygen to supply the needs of the organs to which it is carried.

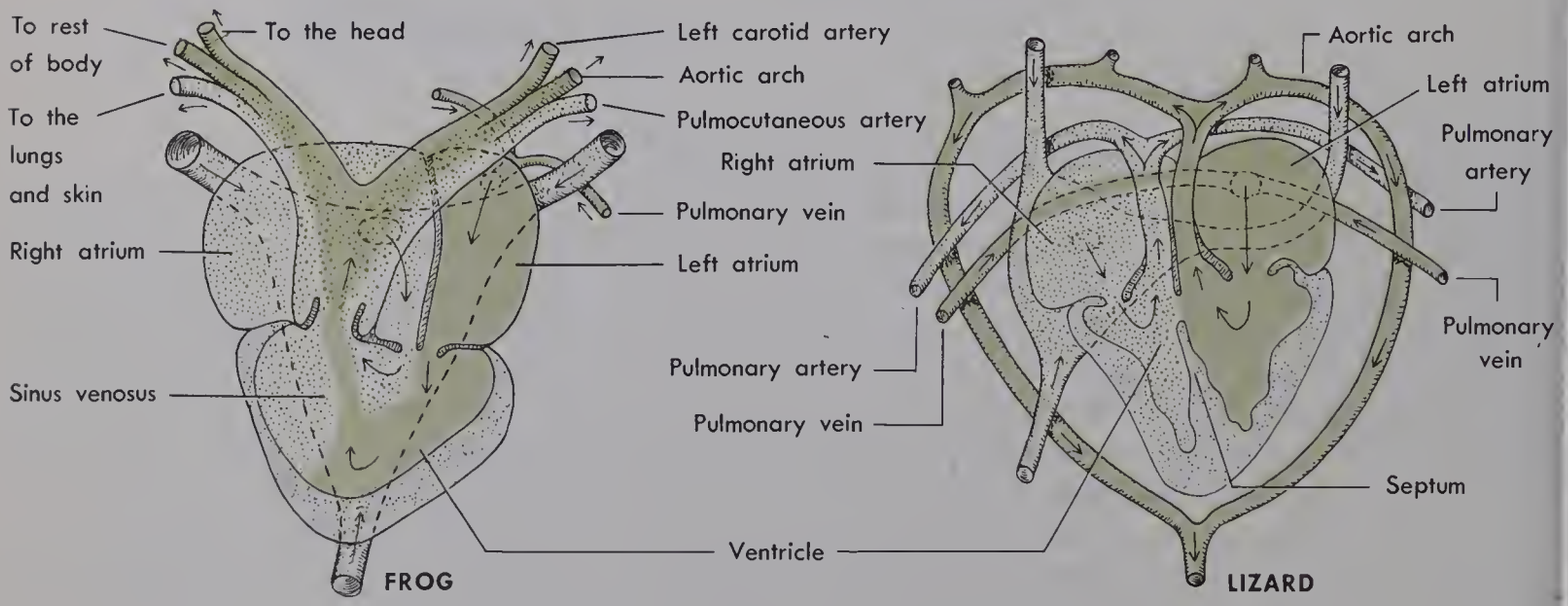


Fig. 13.5 Comparison of an amphibian heart (left) and a reptile heart (right). No mixing of oxygen-deficient blood (stippled) and oxygen-rich blood (light color) occurs in the reptile heart.

Note that the problem created by pressure drop in the capillaries has been solved by the arrangement of the frog circulatory system. Both the gas-exchange organs *and* the interior tissues of the body receive blood under full pressure from the contraction of the ventricle.

During contraction, the ventricle of the lizard heart is divided into two separate chambers

Reptiles have a further modification of the heart. In the lizard, a muscular **septum** partially divides the ventricle (Fig. 13.5). When the ventricle contracts, the opening in this septum closes and the ventricle is momentarily divided into two entirely separate chambers. This prevents mixing of the two bloods. The left half of the ventricle pumps oxygen-rich blood (received from the left atrium) to the body. The right half of the ventricle pumps the oxygen-deficient blood (received from the right atrium) to the lungs.

13.7 FOUR CHAMBERS: BIRDS AND MAMMALS

Birds and mammals use two pumps to circulate their blood

In the hearts of birds and mammals, the septum is complete. This provides two entirely separate pumps. The right atrium receives oxygen-deficient (deoxygenated) blood from the body, and the right ventricle pumps this blood forcibly to the lungs, where it gives off carbon dioxide and picks up a fresh supply of oxygen. This oxygenated blood then returns to the left atrium, passes into the left ventricle, and is pumped out forcibly to all the other organs and tissues of the body. It is probably no coincidence that the only two groups of animals to develop warm-bloodedness (homeothermy) are the birds and mammals, with their two separate circulatory systems. One system is responsible for gas exchange with the environment; the other is responsible for gas exchange with the tissues. The efficiency provided by having these separate systems makes possible the high rate of cellular respiration upon which homeothermy depends.

THE PATHWAY OF CIRCULATION IN MAN

13.8 THE HEART

The right side of the heart

The heart is located roughly in the center of the chest cavity. It is surrounded by a protective membrane, the **pericardium** (Fig. 13.6). Deoxygenated blood from the body enters the right atrium. From here it flows through the **tricuspid valve** into the right ventricle. The name *tricuspid* refers to the three flaps of tissue that guard the opening between the right atrium and the right ventricle. Contraction of the ventricle then closes the tricuspid valve but forces open the pulmonary valve at the entrance to the

The pulmonary system

pulmonary artery. Blood enters the pulmonary artery, which immediately divides into right and left branches leading to the right and left lungs, respectively. These arteries branch and rebranch to form **arterioles.** The arterioles supply blood to the capillary networks of the lungs. Here the blood gives up carbon dioxide and takes on a fresh supply of oxygen. The

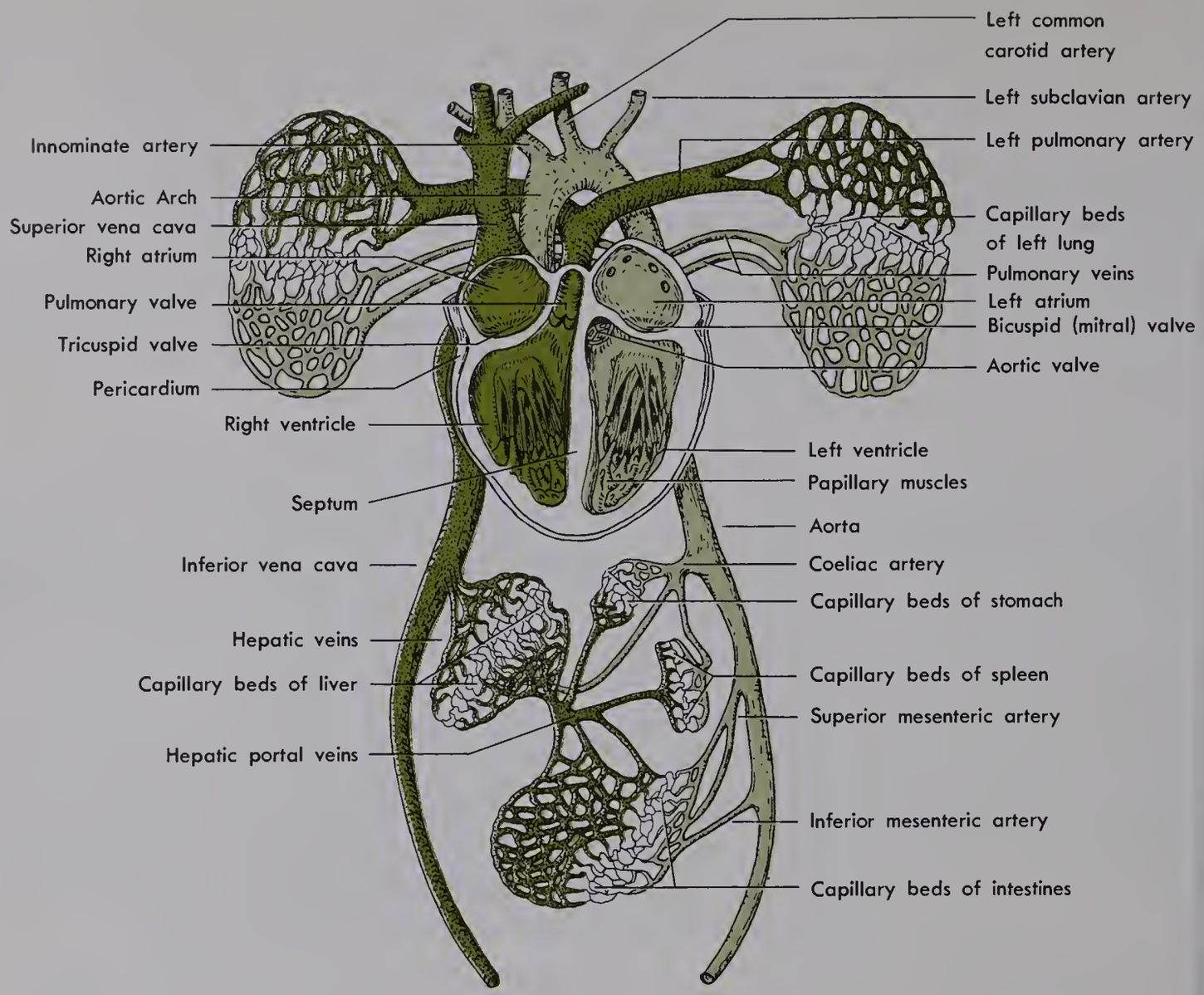


Fig. 13.6 The human heart with a schematic view of the pathway of the blood through the lungs and the viscera. Oxygenated blood is shown in light color, deoxygenated blood in dark color.

capillary networks of the lungs are drained by vessels called **venules.** These serve as the tributaries of the **pulmonary veins.** Four pulmonary veins (two from each lung) carry the oxygenated blood to the left atrium of the heart. This completes the portion of the circulatory system that is known as the **pulmonary system.**

The left side of the heart

From the left atrium the blood passes through the **bicuspid valve** into the left ventricle. Contraction of the ventricle closes the biscuspid value and opens the aortic valve at the entrance to the **aorta.** The first branches from the aorta occur just beyond the aortic valve. Two openings lead to the right and left **coronary arteries** which supply blood to the heart itself.

The coronary system

Although these coronary arteries arise where the aorta is still within the mass of the heart, they pass directly out to the surface of the heart and

extend down across it. They lead to arterioles which in turn supply blood to the network of capillaries that penetrate every portion of the heart. These capillaries are drained by venules which lead to two coronary veins that empty into the right atrium. This portion of the circulatory system is known as the **coronary system.**

Its importance can be seen from the fact that the heart muscle must have a continuous supply of oxygen in order to continue beating. Anything which interferes with the proper operation of the coronary system will have drastic, and often fatal, effects upon the organism. Unfortunately, the coronary system is especially liable to malfunctions. The coronary arteries arise at the point of maximum blood pressure in the circulatory system. The continual high pressure to which they are subjected all too frequently causes a premature wearing of these vessels. This may take the form of a loss of elasticity in the arterial walls, which reduces the amount of blood that can surge through the coronary arteries and hence diminishes the oxygen supply to the heart. Frequently, fatty deposits accumulate on the interior wall of the coronary arteries reducing their bore and thus the amount of blood that they can carry. In either case, the oxygen supply of the heart may be inadequate in times of stress. Painful symptoms of **angina pectoris** result.

Heart attacks

Fatty deposits in a coronary artery or arteriole may also lead to the formation of blood clots. These solid masses stop the flow of blood through the vessel and the capillary network it supplies. This is called a **coronary occlusion,** or heart attack. That portion of the heart whose blood supply is cut off quickly dies of oxygen starvation. If the area is not too extensive, the remaining portions of the heart can, in time, compensate for the damage. All too often, however, the heart fails and death follows. Some 40% of the deaths of adult American males are caused by diseases of the heart.

13.9 THE SYSTEMIC BLOOD VESSELS

The remainder of the circulatory system of man is referred to as the systemic or body system. Figure 13.7 shows the path of the major branches of this system. Blood from the aorta passes into various major arteries which lead to all portions of the body. The blood moves because of the force exerted by the contraction of the left ventricle. The surge of blood which occurs at each contraction is transmitted through the muscular, elastic walls of the entire arterial system, where it can be detected as the **pulse.** Even during the moments when the heart is relaxed (diastole) there is a definite pressure in the arterial system. When the heart contracts (systole), the pressure increases.

Blood pressure

The measurement of blood pressure is a common clinical test. This measurement is always expressed as a fraction, e.g. 120/80. The numerator of the fraction represents the pressure of the arterial blood during systole. The unit of measure is the **torr,** in this example the pressure equivalent to that produced by a column of mercury 120 mm high. The denominator

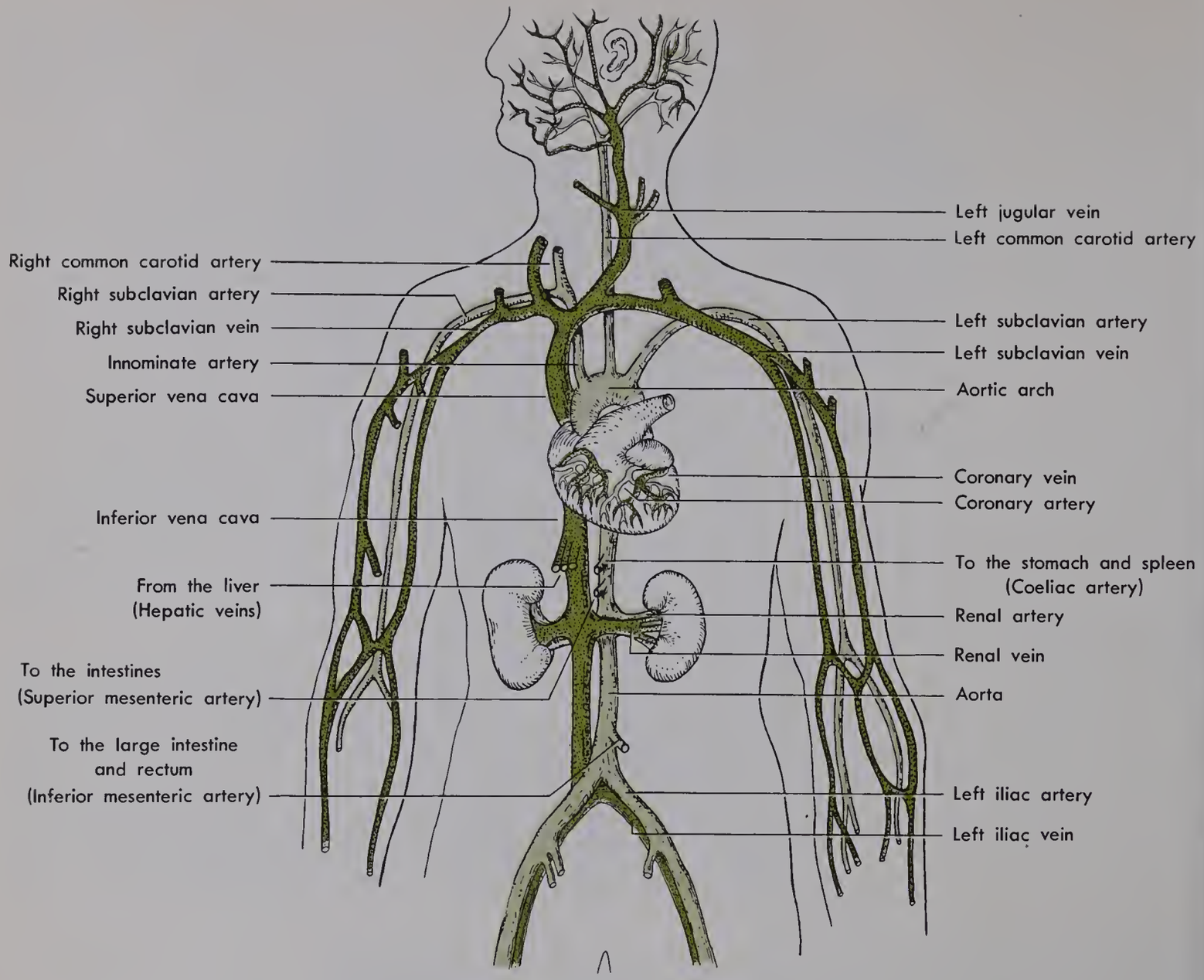


Fig. 13.7 Major blood vessels in the human. Arteries are shown in light color. Veins are indicated in dark color.

represents the pressure during diastole. Although blood pressure varies considerably in a given individual at different times, continual high pressures may be the symptom or cause of a variety of ailments.

When an artery is severed, the blood flows out in spurts because of the pressure exerted by the left ventricle. Severe bleeding may result. Fortunately, arteries are apt to lie somewhat deeper in the body tissues than veins and hence are not so likely to be damaged. If serious arterial bleeding should occur, however, it is important that pressure be applied on the side of the break *nearest* the heart.

13.10 THE CAPILLARIES

The pressure of the arterial blood is largely dissipated when the blood enters the capillaries. Capillaries are tiny vessels whose diameter is just about that of a single red blood cell (7.5 μm). Hence these must pass through the capillary in single file (Fig. 13.13). Although the diameter of a single capillary is quite small, the number of capillaries which arise from a single arteriole is sufficiently great that the total cross-sectional area available for the flow of blood is increased. It has been estimated that there are 60,000 miles of capillaries in the adult human.

The walls of the capillaries consist of a single layer of epithelial cells. Through these walls all the exchanges of materials between the blood and the tissues take place. Although networks of capillaries are not so conspicuous as the heart and major vessels, it is in these networks that the exchange functions of the circulatory system are carried out. In a real sense, the heart and major blood vessels are just accessory parts of the circulatory system. They simply serve to supply blood to and remove blood from the vast network of capillaries.

It has been estimated that the total surface area available for exchanges in the capillary networks is roughly one acre (43,560 ft^2). And, in addition to impressive length and area, the capillary networks (or "beds" as they are often called) of the human body have considerable volume. In fact, their total volume is roughly the same as the total volume of blood (about 5 liters) in the body. If, then, the heart and major vessels are to be filled with blood at all times, it is apparent that all of the capillaries *cannot* be filled at once. Indeed, this is the case. There is a continual redirecting of blood flow from organ to organ in accordance with the changing needs of the body. During vigorous exercise, for example, capillary beds in the skeletal muscles open at the expense of those in the viscera (e.g. intestines). The reverse is true after a heavy meal.

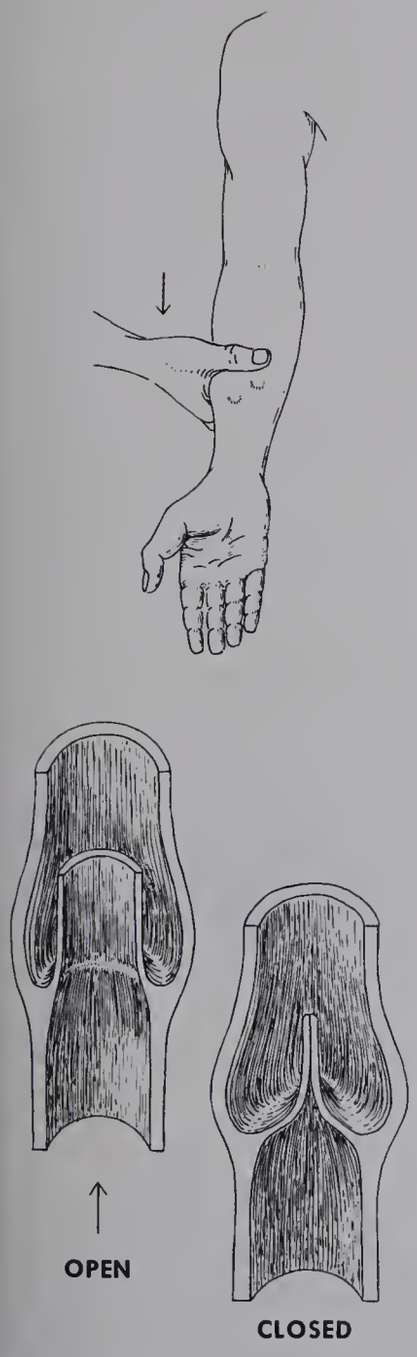


Fig. 13.8 Demonstrating valves in the arm veins. Forcing blood back against a closed valve causes the vein to bulge at that point.

13.11 RETURN OF BLOOD TO THE HEART

When the blood leaves the capillaries and enters the veins, there is little pressure to force it along. Blood in the veins below the heart is helped back up to the heart by the **muscle pump.** This is simply the squeezing effect that active muscles have upon the veins running through them. Of course, this squeezing effect would be useless if there were not some mechanism to insure that the blood travels in one direction. This mechanism is a system of valves that permit venous blood to flow towards the heart but prevent backflow. You can demonstrate these valves for yourself by firmly stroking the veins near the inner surface of your forearm. If you stroke away from the heart, you will force the blood back against the valves. They will close and a bulge will appear at that point (Fig. 13.8). (The walls of veins are quite thin and flabby.)

The importance of the muscle pump is dramatically illustrated when soldiers faint after standing "at attention" for long periods of time. The

loss of consciousness results from an insufficient supply of blood (and hence of oxygen) to the brain. This develops because of a gradual accumulation of blood in the legs as a result of inactivity of the leg muscles. Fainting is a very nice self-protective mechanism in these cases. As the body moves from the vertical to the horizontal position, the heart reaches the same level as the legs. The blood in the legs no longer has to return several feet against gravity, and soon proper circulation is re-established. Fainting is more apt to occur among recruits than among seasoned soldiers. The latter know that unlocking the knees slightly and inconspicuously flexing the calf muscles will prevent fainting. These actions aid in maintaining the flow of venous blood back to the heart.

Importance of the muscle pump

The development of high-speed aircraft (including space capsules) has posed a number of problems of human physiology for the occupants. One of these is the gravity (or "G") force set up in pulling out of a dive or on leaving and re-entering the earth's atmosphere. These G forces can seriously impede the return of blood from the lower portion of the body to the heart and cause fainting or "blackout" at a very critical time. This problem has been solved by the development of anti-G suits. The walls of these suits are inflated with compressed air when the number of G's begins to build up. The pressure exerted on the body (and hence on the veins, which generally lie quite close to the surface) forces the blood back to the heart in opposition to the G forces.

Because of the modest forces involved in getting blood through the veins, the flow is quite even. Blood leaves a cut vein in a smooth, steady stream. For this reason and because the veins lie near the surface, it is relatively easy to check the bleeding. This must be done, however, by applying pressure on the side of the cut away from the heart.

It is a common error to think of arteries as the blood vessels that carry oxygenated (bright red) blood and the veins as the vessels that carry deoxygenated (dark red) blood. While this is usually the case, there is one striking exception in humans. Can you think of what it is? Arteries must therefore be defined as those vessels that carry blood *away from* the heart. Veins are those vessels that carry blood *toward* the heart.

BLOOD

The medium of transport in the circulatory system is blood. Not only does the blood transport oxygen and carbon dioxide to and from the tissues and lungs, but it also transports other materials throughout the body. These include food molecules (e.g. glucose, amino acids), metabolic wastes (e.g. urea), ions of various salts (e.g. Na^+, Ca^{++}, Cl^-, HCO_3^-), and the hormones. Blood also serves to distribute heat in the body. In addition to its function as a transport agent, blood plays an active part in combating infective disease agents (e.g. certain bacteria) that gain access to the body.

Functions of the blood

13.12 THE BLOOD CELLS

Blood is a tissue

Blood is a liquid tissue. It consists of cells (and cell fragments) suspended freely in a watery medium, the plasma. The cells and cell fragments constitute the so-called "formed" elements of the blood. They are sufficiently large that they can be observed under the light microscope. There are three types of "formed" elements: the red blood cells, or erythrocytes, the white blood cells, or leucocytes, and the platelets, or thrombocytes.

1. The **red blood cells** (RBC's) are the most numerous of the three types. Normal women possess about 4.5 million of these cells in each cubic millimeter of blood. In normal men, the average runs somewhat higher: about 5 million. However, these values can fluctuate over a considerable range, depending upon such factors as the altitude at which the individual lives (Peruvians living at an altitude of 18,000 feet may have values as high as 8.3 million) and the individual's state of health.

The RBC's are disk-shaped. They have a diameter of 7.5 μm and a thickness at the rim of 2 μm. The center of the disk is thinner (1 μm) than the rim. This interesting "biconcave" shape speeds up the exchange of gases between the cell and the plasma.

The life of red blood cells

In the adult, the RBC's are produced by special cells (Fig. 13.9) located in the marrow of bones, particularly in the ribs, sternum (breast bone), and vertebrae. When first formed, the RBC's have a nucleus and not very much hemoglobin. However, as they mature, the quantity of hemoglobin in the cell increases until some 280 million molecules—representing about 90% of the dry weight of the cell—are present. Toward the end of this process of hemoglobin synthesis, the nucleus is squeezed out of the cell.

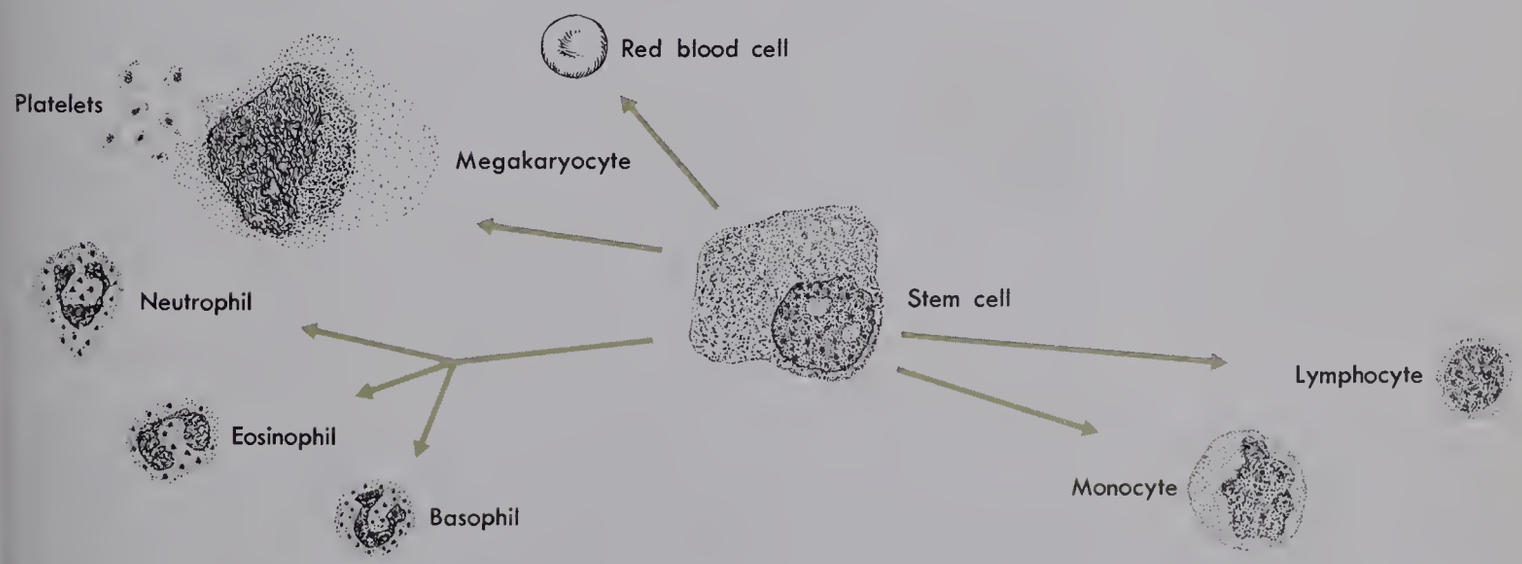


Fig. 13.9 The cells of the blood. Red blood cells transport oxygen and carbon dioxide; platelets participate in blood clotting; and the rest of the cells aid the body in combating infection. It is still uncertain whether the cells on the right share a common stem cell with those on the left. All of the blood cells are manufactured in the bone marrow, although lymphocytes and monocytes are produced in other locations (e.g. lymph nodes) as well.

The life span of these cells is about 120 days. The death of a red blood cell occurs when its cell membrane ruptures as the cell is squeezed through a capillary. The fragments of the ruptured cell are then ingested by endocytic cells present in the liver and in a sac-shaped structure called the spleen. Most of the iron of the hemoglobin is reclaimed for re-use. The remainder of the hemoglobin molecule is broken down. Some of the breakdown products—the bile pigments—are excreted by the liver in the bile. It has been estimated that three million RBC's die and are scavenged by the liver and spleen every second.

The continual loss of RBC's is normally compensated for by the action of the bone marrow. In fact, healthy bone marrow can, when necessary, produce RBC's at four or more times the normal rate of cell destruction. Thus, after severe bleeding (or blood donations), the bone marrow quickly brings the RBC content of the blood back to normal.

Anemia

Under some circumstances, the rate of RBC loss exceeds the rate of RBC formation. When this occurs, the concentration of RBC's in the blood decreases, a form of **anemia.**

There are five kinds of white blood cells

2. The **white blood cells** (WBC's) are much less numerous than the red, the ratio between the two types being approximately 1:700. There are actually five distinct types of WBC found in the circulating blood (Fig. 13.9). All possess nuclei. They range in size from the lymphocytes, which are not much larger (10 μm) than RBC's, to the monocytes, which may be three times as large (25 μm). The shape of the WBC's is quite variable, especially when they are being carried through the capillaries.

Neutrophils and monocytes engulf foreign particles

The general function of the WBC's is to protect the body from infection. The **neutrophils** and **monocytes** accomplish this by ingesting, by endocytosis, foreign particles (e.g. bacteria) that get into the body. To carry out this function these leucocytes, in response to a chemical attractant, squeeze themselves through the capillary walls in areas where tissue damage is occurring. Once free in the tissues, they begin endocytosis. The bacterium or other particle is engulfed in a vacuole. This fuses with a lysosome, the enzymes of which may destroy the bacterium. The granules which are so abundant in the cytoplasm of neutrophils are the lysosomes. As endocytosis proceeds, their number decreases rapidly. Usually endocytosis ends with the death of the WBC. An accumulation of dead WBC's and products of tissue destruction is called pus.

The life span of neutrophils is relatively short. Even in a healthy person, they die within a few days. You might well ask why these cells die so soon if there is no active infection to combat. The answer is that they are constantly engaged in the control of bacteria that are permanent residents in our mouths, air passages, large intestine (see Section 7.11), and elsewhere. Generally, this control is sufficiently good that we can tolerate the presence of these bacteria. However, a variety of stresses may temporarily lower our resistance and permit these organisms to invade our tissues. A dramatic illustration of this is the disease *agranulocytosis.* In this disease, the production of new leucocytes ceases. Heavy exposure to radia-

Agranulocytosis

tion, such as accompanies an atomic bomb blast, is one of several causes of agranulocytosis. With leucocyte production stopped, the bacteria normally held in check in our bodies soon begin to multiply. After two days, ulcers of the mouth and large intestine, or serious lung infection, result. These infections soon spread throughout the body. Unless treatment with antibiotics is started quickly, death follows within a few days. Agranulocytosis is one more peril in our atomic age. It is also a fine illustration of the importance of our leucocytes in maintaining an uneasy truce with the various species of bacteria we harbor within our bodies.

Lymphocytes are necessary for the development of immunity

Lymphocytes generally do not carry on endocytosis. They do, however, combat disease by participating in the formation of **antibodies.** Antibodies are proteins that are produced whenever foreign macromolecules enter the body. Such foreign molecules are called **antigens.** Foreign protein, polysaccharide, and nucleic acid molecules can all act as antigens.

The relationship between antigens and antibodies is very specific. Each antigen stimulates the production of antibody molecules that are capable of combining directly with that antigen and, generally, no other. When antigen and antibody do combine, the resulting complex has properties different from those of its components. For example, the complex may settle out of solution and/or be more easily engulfed by endocytic cells.

An invading parasite, such as a bacterium or a virus, has on its surface macromolecules that are foreign to the host and hence antigenic. The ability to manufacture antibodies against these antigens and thus deactivate them is clearly an important weapon against invasion by disease-producing organisms. The result is immunity. The role that lymphocytes play in the immune response will be discussed in Chapter 30.

Eosinophils and basophils

The percentage of **eosinophils** and **basophils** in the blood is normally very low. Their function is not yet fully understood although there is evidence that each plays a role in combating disease. The number of eosinophils rises markedly in certain diseases, especially those caused by parasitic worms. The number of basophils also increases during infections. Those at the site of infection appear to release substances (e.g. histamine) that increase the flow of blood in the area. Although there is a great deal yet to be learned about the functions of the various white blood cells, it seems fairly certain that each in its own way contributes to the body's defense against disease.

Leukemia

All the white blood cells are manufactured in the bone marrow. Rarely, a precursor cell becomes cancerous. As a result, the number of one or more kinds of leucocytes becomes far greater than normal, a condition known as **leukemia.** A variety of harmful symptoms (often including severe anemia) follow. In some forms of the disease, medical care can slow down the development of symptoms and prolong the patient's life for many years. But as yet there is no cure.

Platelets are essential for blood clotting

3. The **platelets** are cell fragments produced by large cells (megakaryocytes) in the bone marrow. Platelets are disk-shaped and much smaller (2

μm) than the RBC's. Normally, there are 150,000–400,000 platelets in each cubic millimeter of blood. They are very important in the process of blood clotting (Section 13.21).

13.13 THE PLASMA

Composition of the plasma

The fluid in which the blood cells are suspended is a straw-colored liquid called plasma. Figure 13.10 shows the chemical composition of the plasma. Most of it is water. Water is an excellent solvent and it is this property that makes it such an excellent transport medium. A variety of molecules and ions are found dissolved in the water of the plasma. They are transported by the blood from an exchange organ (e.g. intestine) or reserve supply (e.g. liver for glucose, bone for Ca^{++}) to the tissues that need them.

Chemical composition of blood plasma

Component	Percent	
H_2O	90	
Inorganic salts	less than 1	
Major proteins	7	—(serum albumin 4%, serum globulin 2.7%, fibrinogen 0.3%)
Other substances (foods, wastes, hormones, etc.)	2	

Fig. 13.10

In addition to the small molecules and ions in solution, the plasma contains a substantial quantity (7%) of proteins. There are three major protein fractions in the plasma.

The plasma proteins

1. Serum albumin. Serum albumin is manufactured by the liver. It aids in the maintenance of blood volume and hence blood pressure. The way in which this effect is achieved is discussed below.

2. Serum globulin. This protein fraction is not homogeneous; that is, there are many specific proteins in the blood which are collectively referred to as serum globulins. Some of these are enzymes and some, the gamma globulins, are circulating antibodies. Perhaps you have received at some time an injection of gamma globulin to give you temporary protection against measles or polio.

3. Fibrinogen. Fibrinogen is an essential component of the clotting process (Section 13.21).

In addition to their specialized functions, all the plasma proteins serve as buffers. Their presence in the plasma suppresses any marked change in its pH. The proteins can combine with both acids and bases, and in so doing, prevent drastic shifts in the acid-base balance of the plasma.

Functions of the Blood

Defense against infection

Transport of materials

The two major functions of the blood are: (1) to transport materials to and from all the tissues of the body and (2) to defend the body against infectious disease. The second function was mentioned in the discussion of the white blood cells and will be considered further in Chapter 30. The first function concerns us here. It is the great solvent power of the water in the plasma that makes blood such an effective transport medium. Glucose, amino acids, short-chain fatty acids, glycerol, vitamins, hormones, nitrogenous wastes (e.g. urea), and many ions are all transported dissolved in the plasma of the blood. Only in the case of gas transport is the plasma incapable of handling the job alone. It is the RBC's that carry out the bulk of oxygen and carbon dioxide transport.

13.14 OXYGEN TRANSPORT

As much as 90% of the dry weight of a red blood cell consists of the red pigment **hemoglobin.** Hemoglobin (Hb) is a protein consisting of four polypeptide chains to each of which is attached a prosthetic group, *heme* (Fig. 8.5). At the center of each heme is an atom of iron.

Hemoglobin combines reversibly with oxygen

Oxygen and hemoglobin combine readily, one molecule of oxygen combining with each heme group. Thus the presence of Hb in the bloodstream greatly increases the amount of oxygen that can be carried by it. The affinity of Hb for oxygen is, however, only part of the story of oxygen transport. Many substances combine vigorously with oxygen. What makes hemoglobin useful as an oxygen transport agent is that it releases the oxygen again to the tissues. In other words, the reaction between hemoglobin and oxygen is freely reversible (Fig. 13.11). Under the conditions of temperature, pH, and increased oxygen pressure existing in the capillaries of the lungs, the reaction proceeds to the right. The purple-red hemoglobin of the venous blood becomes converted to the bright red oxyhemoglobin of the arterial blood. Under the conditions of temperature, pH, and reduced oxygen pressure that exist in the capillaries of the tissues, the reverse reaction is promoted and the oxyhemoglobin gives up its oxygen. Actually, the reverse reaction in the tissues never becomes completed. One hundred ml of arterial blood carries about 20 ml of oxygen. (Of this only 0.3 ml is carried in solution in the plasma; all the rest is carried in the RBC's.) In the resting human, only about one-fourth (5.3 ml) of this oxygen is released to the tissues. About 5000 ml of blood are pumped every minute by a

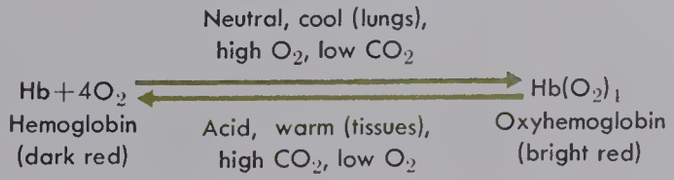


Fig. 13.11 Reversible reaction between oxygen and hemoglobin.

resting human and hence about 275 ml of oxygen are transported from the lungs to the tissues in that time.

During vigorous exercise, as much as three-fourths of the oxyhemoglobin in the tissues gives up its oxygen. Furthermore, the heart of a trained athlete may pump seven or eight times as much blood during exercise as during rest. Thus the amount of oxygen delivered to the tissues may increase up to 5775 ml per minute or over 20 times that delivered during rest.

Such a performance would be impossible without the presence of the RBC's. One hundred ml of plasma alone can carry only 0.3 ml of oxygen. About 0.18 ml of this dissolved oxygen is released in the tissues. A quick calculation will show that a human depending on plasma alone for oxygen transport would have to pump 30 times as much blood every minute, even when at rest, to satisfy the demands of his tissues.

Carbon monoxide poisoning

The importance of the loose, reversible association between hemoglobin and oxygen is dramatically illustrated in carbon-monoxide poisoning. Carbon monoxide, like oxygen, combines readily with hemoglobin, but, in so doing, prevents the Hb from also combining with oxygen. Carbon monoxide is not, however, released readily by the hemoglobin. Thus an individual exposed to carbon monoxide (such as in a closed garage with a running automobile engine) soon has more and more of his hemoglobin inactivated for the transport of oxygen. The affinity of Hb for carbon monoxide is 230 times that for oxygen. Hence, in air containing just 1/230 as much carbon monoxide as oxygen, the carbon monoxide competes on equal terms with the oxygen. One-half of the hemoglobin in the bloodstream will become incapable of transporting oxygen. The skin of the victim of carbon-monoxide poisoning develops a cherry-red color because this is the color which hemoglobin becomes when it combines with carbon monoxide. The prompt removal of the victim from the fouled atmosphere and the application of oxygen may save his life.

13.15 CARBON DIOXIDE TRANSPORT

Carbon dioxide is much more soluble in water than is oxygen. One reason for this is that carbon dioxide combines chemically with water, forming carbonic acid,

$$CO_2 + H_2O \rightleftharpoons H_2CO_3.$$

This then dissociates into a hydrogen ion (H^+) and a bicarbonate ion,

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-.$$

The red cells also transport CO_2

If all the carbon dioxide transported by our bloodstream were simply carried in the plasma this way, the pH of the blood would be lowered from its normal level of pH 7.41 to about pH 4.5. This would be instantly fatal. However, not more than 5–10% of the carbon dioxide produced by the tissues is actually transported in this fashion. The red blood cells accom-

plish the transport of the rest. Forty to forty-five percent of the carbon dioxide actually combines with hemoglobin in the RBC's, forming carbaminohemoglobin. The carbon dioxide probably does not join the hemoglobin at the same place on the molecule that oxygen does, but the release of oxygen from hemoglobin in the tissues does increase somewhat its ability to carry carbon dioxide. Similarly, in the lungs, the pickup of oxygen by hemoglobin promotes the release of carbon dioxide from it.

As carbaminohemoglobin

The RBC's assist in the transport of carbon dioxide in still another way. When carbon dioxide enters the red blood cell, some of it combines with water within the cell, forming carbonic acid. The reaction is greatly speeded by an enzyme, carbonic anhydrase, found within the cell. (Fortunately, this enzyme is not present in the plasma and, thus, as we have seen, not much carbonic acid is found there.) The hydrogen ions released by the carbonic acid then combine with the protein portion of hemoglobin. Thus combined, they do not lower the pH. Most of the remaining bicarbonate ions then diffuse back out into the plasma. When the RBC's reach the lungs, these reactions are reversed and carbon dioxide is released to the alveolar air. Fifty percent of the carbon dioxide transported by the blood is carried in this fashion. Thanks to these two important mechanisms provided by the red blood cells, we are able to transport carbon dioxide quickly and safely from the tissues to the lungs.

As bicarbonate ions

13.16 EXCHANGES BETWEEN THE BLOOD AND THE CELLS

The medium of transport in the human body is blood. Man's circulatory system is a closed one, however, and blood does not come in direct contact with the cells. (An exception, of course, is the epithelial cells that line the walls of the arteries and veins and make up the walls of the capillaries.) The number and distribution of capillaries is such that probably no cell is ever farther than 50 μm from a capillary (Fig. 13.12). Nevertheless, materials must cross this gap between the blood and the cells. This is accomplished, in large measure, by diffusion. However, some blood components also flow out of the capillaries. When blood enters the arteriole end of a capillary, it is still under the pressure (about 35 torr) produced by the contraction of the ventricle. As a result of this pressure, some filtration of the blood through the walls of the capillaries takes place. Between the epithelial cells that make up the wall of the capillary are tiny pores. It is through these pores that filtration of the blood occurs. The pores are sufficiently small that no formed elements get through (except when migration of WBC's occurs). However, a substantial amount of water and some of the proteins in the blood plasma pass through the capillary wall and into the cell-containing region, the tissue space (Fig. 13.13). This fluid, called **interstitial fluid,** is simply blood plasma minus most of the proteins. In the tissue space, it bathes the cells. Any substances present in this fluid can then pass into the cells by diffusion or active transport. Also, any materials present in excess amounts in the cells (e.g. carbon dioxide or nitrogenous wastes) can diffuse out into the interstitial fluid.

Formation of interstitial fluid

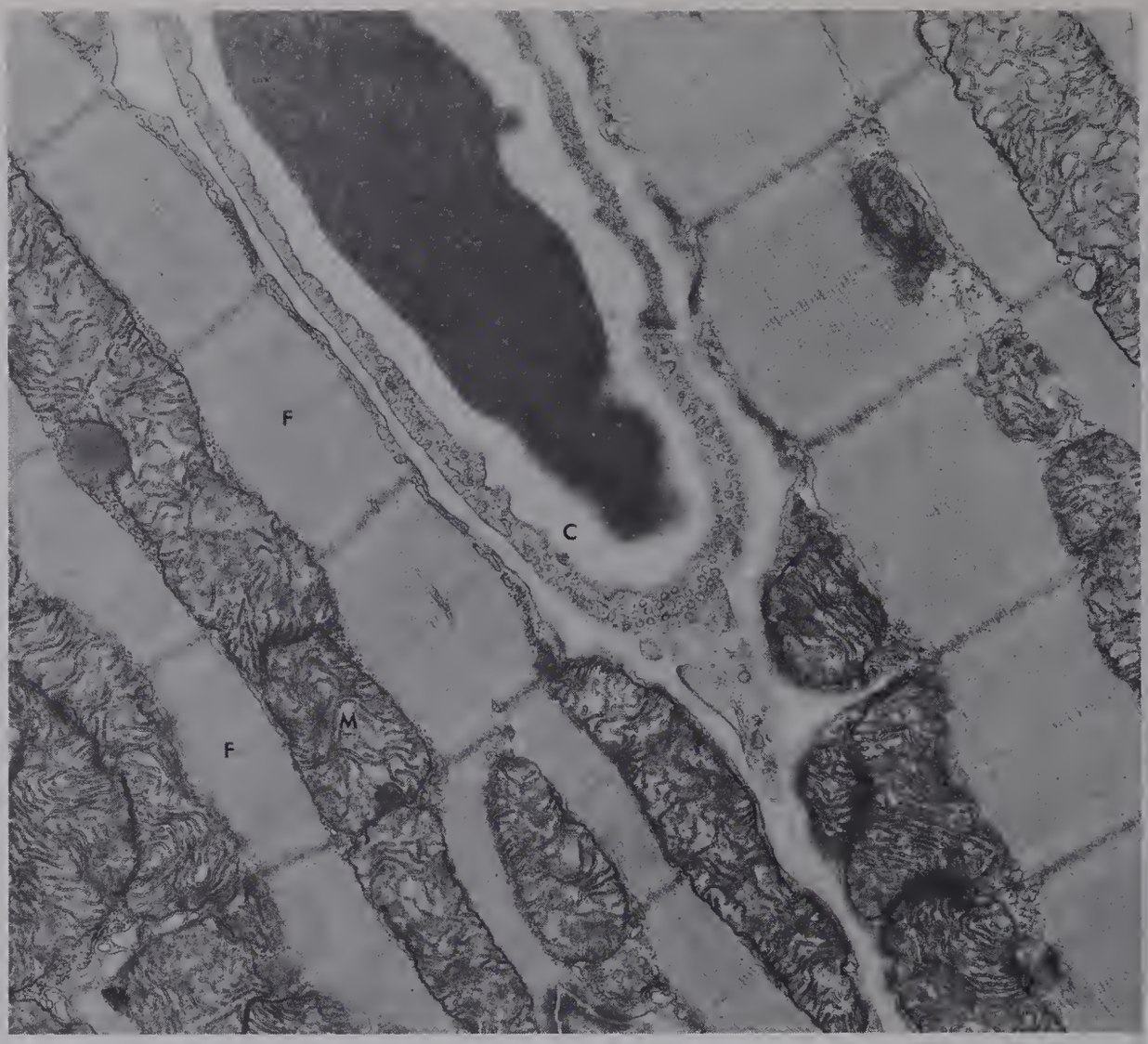


Fig. 13.12 Capillary between two adjacent heart muscle cells (17,000 X). Rows of mitochondria (M) can be seen between the contractile units (F). Note the thinness of the epithelial cell that forms the wall of the capillary (C). The object within the capillary is a red blood cell. (Reproduced with permission from Keith R. Porter and Mary A. Bonneville, *An Introduction to the Fine Structure of Cells and Tissues,* 2nd. ed., Lea and Febiger, Philadelphia, 1964.)

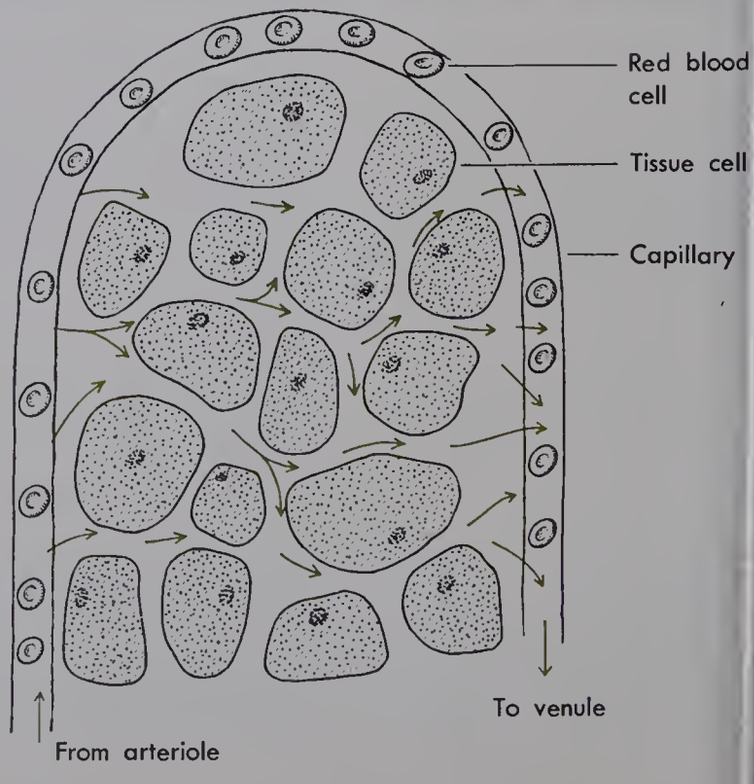


Fig. 13.13 Mechanism of exchange between blood and cells. The cells are bathed in interstitial fluid which is filtered from the blood.

Near the venous end of a capillary, the blood pressure is greatly reduced (about 15 torr). Here, another force comes into play. Although the composition of interstitial fluid is similar to that of blood plasma, it does contain a smaller quantity of proteins than plasma and, thus, a somewhat larger quantity of water. Because of this difference, an osmotic pressure is established. The osmotic pressure is quite small (about 25 torr) but it is greater than the blood pressure at the venous end of the capillary. Consequently, the fluid reenters the capillary here.

Return of interstitial fluid to the blood

It is interesting to note that most capillaries curve around a tissue space in a horseshoe shape. Thus fluid formed at the arteriole end of a capillary flows across the tissue space, bathing the cells, and re-enters the same capillary at the venule end. Where this structural arrangement is not found, the venule end of some other capillary is located across the tissue space.

13.17 THE LYMPHATIC SYSTEM

From one-third to two-thirds of the plasma entering a capillary passes into the tissue space. Although most of this returns to the venule end of the capillary, some does not.

The small amount of fluid that remains is apt to be particularly rich in serum proteins which filtered through the capillary walls. These do not pass back into the capillary by diffusion. Fortunately, the bloodstream does not lose this fluid and protein for very long. Excess fluid in the tissue spaces is picked up by tiny vessels called lymph capillaries. These are very porous and easily pick up the proteins present. (In Chapter 7 we studied the lymph capillaries in the villi: the lacteals.) The lymph capillaries lead into still larger vessels which make up the lymphatic system. The flow through the lymph vessels is quite slow. It is accomplished in a manner similar to that of venous blood. Muscular activity compresses the lymph vessels and squeezes the fluid, now called **lymph,** along. The lymph can flow in only one direction because of the many valves present.

Some interstitial fluid enters the lymphatic system

All the lymph collected from the entire left side of the body, plus the lymph collected from the digestive tract and the right side of the lower part of the body, flows into a single major lymph vessel, the **thoracic duct.** This duct empties about 100 ml of lymph every hour into the left subclavian vein (Fig. 13.14). The lymph produced in the right side of the head, neck, and chest is collected by the right lymph duct and empties into the right subclavian vein. In this way, lymph with its content of protein is returned to the bloodstream.

A number of things may upset the normal production or flow of lymph. An increase in blood pressure in the capillaries or a decrease in plasma proteins (such as may follow prolonged malnutrition) will result in the production of abnormally great quantities of lymph. (Why?) The lymphatic system may be unable to handle the increased lymph production successfully, and the lymph will begin to accumulate in the tissues and to distend them. This condition is known as **edema.** Another cause of edema

Edema

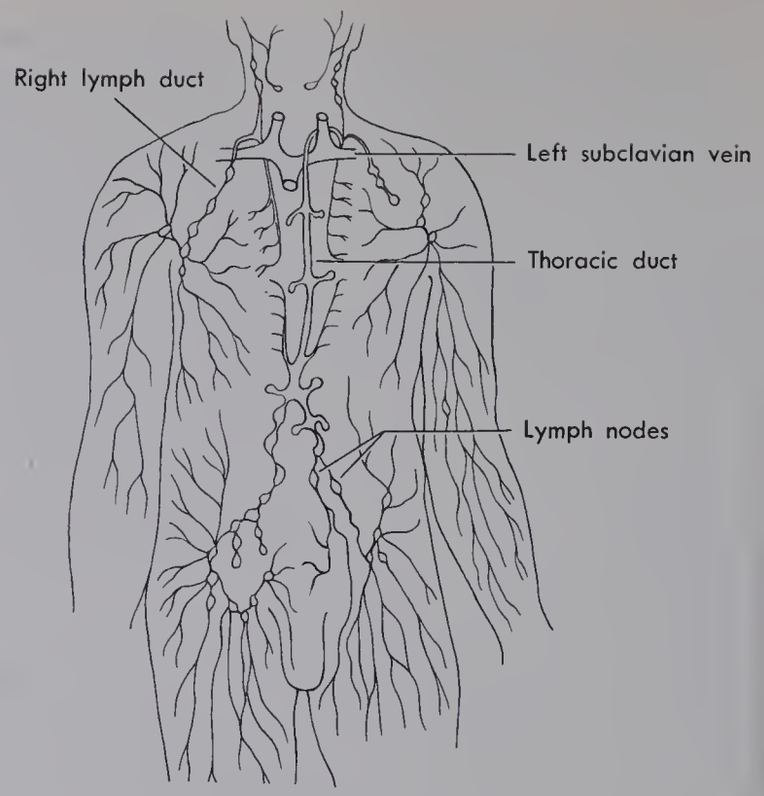


Fig. 13.14 Lymphatic system of man. It returns valuable plasma proteins to the blood and aids in combating infection.

Fig. 13.15 Elephantiasis. A heavy infection with filarial worms (nematodes) has so damaged the lymph vessels of one leg that they can no longer drain lymph from it. The resulting chronic edema and thickening of the skin cause increasing deformity. The worms are transmitted from person to person by mosquitoes.



is blockage of the lymph vessels. In the tropics this may occur as a result of infection with a parasitic roundworm, the filarial worm. The resulting edema may cause portions of the body, such as the legs or arms, to become grossly enlarged, a condition known as elephantiasis (Fig. 13.15).

Scattered at various places in the lymphatic system (especially in the groin, armpits, abdomen, and neck) are several hundred lymph nodes (Fig. 13.14). These contain cavities, or sinuses, into which the lymph flows. The walls of the sinuses are lined with endocytic cells which engulf any foreign particles that might be present in the lymph. This mechanism protects the bloodstream from invasion by bacteria. It is one of the important body defenses against infectious disease. When combating a heavy infection, the lymph nodes may swell, resulting in "swollen glands." Lymph nodes also manufacture lymphocytes which then enter the bloodstream at the subclavian veins.

THE CONTROL OF CIRCULATION

Earlier in the chapter we mentioned that the amount of blood pumped by the heart increases with exercise and that the blood supply in the capillary beds varies from time to time over wide ranges. It is not surprising that a system as important as the circulatory system should be flexible in its operation so as to meet the changing needs of the body. This flexibility arises from a well-integrated system of controls.

13.18 THE HEART

The heart itself plays an important role in determining how much blood is pumped in a given period of time. During rest, the heart beats about 70 times a minute in the adult male while pumping about 5 liters of blood. The stimulus that maintains this rhythm is entirely self-contained. Embedded in the wall of the right atrium is a mass of specialized heart tissue called the sino-atrial (S-A) node (Fig. 13.16). The S-A node is often called the **pacemaker** of the heart because it establishes the basic rhythm at which the heart beats. The fibers of heart muscle, like all cells, are charged positively on the outside and negatively on the inside. In the pacemaker this charge breaks down spontaneously about 70 times each minute. This, in turn, initiates a similar breakdown in the nearby muscle fibers of the atrium. A tiny wave of current sweeps over the atria, causing them to contract. When this current reaches the region of insulating connective

The pacemaker

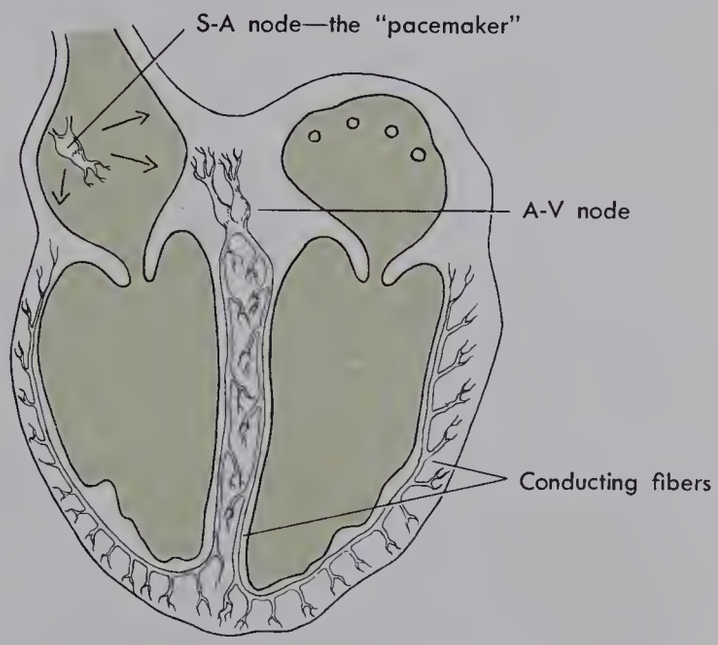


Fig. 13.16 Although nerves run to the heart, its basic rhythm is established by impulses arising in the S-A node.

tissue between the atria and the ventricles, it is picked up by the atrial-ventricular (A-V) node. This leads to a system of branching fibers that carry the current to all parts of the ventricles, which then contract vigorously. The entire operation constitutes **systole.** A period of recovery follows in which the heart muscle and S-A node become recharged. The heart muscle relaxes and the atria fill during this period **(diastole)**.

Ventricular fibrillation

Damage to the pacemaker does not necessarily result in heart failure. Even without the pacemaker, the ventricles can maintain a beat, although it is considerably slower. There is, however, a danger that impulses arising in the ventricles may become disorganized and random. When this happens, the ventricles begin to twitch spasmodically and cease to pump blood. This is known as ventricular fibrillation. Death follows quickly unless immediate measures are taken to start rhythmic beating again. In the past this has meant opening up the chest cavity and massaging the

heart by hand. Obviously such heroic measures have usually been confined to cases of ventricular fibrillation occurring while the patient was under surgery.

Ventricular fibrillation is probably the immediate cause of death in 25% of all cases. Although most cases arise spontaneously in damaged hearts, moderately severe electric shocks can also cause fibrillation. Unfortunately, the 60-cycle alternating current in such widespread use today is especially effective at this.

Artificial pacemakers

If, because of a heart attack or for other reasons, a person seems to be unduly predisposed to fibrillation, he can now be given an artificial pacemaker. This is simply a device that generates rhythmic impulses which are transmitted to the heart by fine wires. Thanks to miniaturization techniques and long-lived batteries, modern pacemakers are now implanted in the body, close enough to the skin to be reached through a small incision when maintenance is needed. The more sophisticated pacemakers are even able to adjust the frequency of their output to correspond to the varying demands placed on the heart as the patient alters the level of his physical activity. It has been estimated that 50,000 Americans are now wearing artificial pacemakers.

13.19 AUXILIARY CONTROL OF THE HEART

Although the pacemaker sets the basic rhythm of the heart, the rate and strength of beating can be modified by other factors. Located in the medulla of the brain are two auxiliary control centers. These initiate impulses which travel to the heart by way of nerves.

The accelerator nerves

Impulses travelling to the heart by way of the *accelerator* nerves cause an increase in the rate and strength of the heartbeat and hence an increase in the rate of flow of blood through the body. These impulses are most likely to arise when the individual is subject to some stress such as fear or violent physical exertion. The heartbeat may increase to 140, or even more, beats per minute. Simultaneously, the strength of contraction increases. The total quantity of blood pumped by the heart may thus increase up to 35–40 liters per minute. Have you ever noticed your heart start to "pound" rapidly when you were frightened? The accelerator nerves are responsible for this.

Vigorous exercise accelerates heartbeat in two ways. As cellular respiration increases, so does the carbon dioxide content of the blood. This stimulates special carbon dioxide receptors located in the carotid arteries and aorta (Fig. 13.7), and these transmit impulses to the medulla for relay, by way of the accelerator nerves, to the heart. Furthermore, as muscular activity increases, the muscle pump (Section 13.11) drives more blood back to the right atrium of the heart. The atrium becomes distended with blood, thus stimulating stretch receptors located in its wall. These, too, send impulses to the medulla for relay to the heart. Working together, these two mechanisms thus ensure that the increased demands on the heart during vigorous exercise are met.

The vagus nerves inhibit the rate and strength of the heartbeat

Two *inhibitory* nerves also run from the medulla to the heart. Impulses passing down these, the **vagus** nerves, cause a slowing down of the heartbeat. Pressure receptors are present in the aorta and in the carotid arteries. When the blood pressure increases above normal levels, these receptors send impulses back to the medulla. The medulla, in turn, relays these through the vagus nerves to the heart. Heartbeat and blood pressure diminish. The vagus nerves thus act antagonistically to the accelerator nerves and protect the heart from unnecessary or excessive overactivity.

13.20 PERIPHERAL CONTROL OF CIRCULATION

Nerves also affect the flow of blood through the arterioles. The walls of these vessels are muscular. Their constriction reduces the bore of the vessels, thus increasing blood pressure but reducing blood flow. Dilation of the vessels has the opposite effect. For example, in times of danger or other stress, the arterioles supplying blood to the skeletal muscles will be dilated while the bore of the vessels supplying the digestive organs will be decreased. This action is carried out by nervous stimulation and also by a hormone, adrenaline, which is released into the blood itself. On the other hand, after a full meal, the blood supply to the digestive organs will be increased at the expense of that to the skeletal muscles.

Regulation of the diameter of the arterioles

Many chemical substances in addition to adrenaline have been discovered in the blood which affect the bore of arterioles and hence blood pressure. The pituitary gland and the kidneys both release substances that constrict arterioles and thus raise blood pressure. On the other hand, cells where infection or other damage is occurring release substances, e.g. *histamine,* that dilate the arterioles and thus increase blood flow in the area. Basophils and other cells participate in the process. Much research remains to be done on the action and interrelationships of these substances.

Although the walls of the capillaries have no muscles and hence cannot be controlled directly, the over-all action of the capillary bed is under precise control. Running between the arterioles and venules is a vessel called the "thoroughfare channel" (Fig. 13.17), from which arise the true capillaries. There is a ring of muscle, the precapillary sphincter, at the point where each capillary branches from the thoroughfare channel. When it is relaxed, blood flows into the capillary. When it is contracted, blood flow through the capillary ceases. Generally, the stimuli which cause constriction of the arterioles in any given portion of the body also cause constriction of the precapillary sphincters. The same is true for stimuli which cause dilation of the arterioles.

Function of the precapillary sphincters

There is also a considerable degree of *local* control over the capillary beds. When cells in a tissue space are metabolizing rapidly, they release substances that relax the precapillary sphincters. This is a very nice control mechanism because rapidly metabolizing cells need additional supplies of glucose and oxygen. They also need to have their wastes carried away more quickly. Both these needs are taken care of by increasing the blood flow through the nearby capillaries.

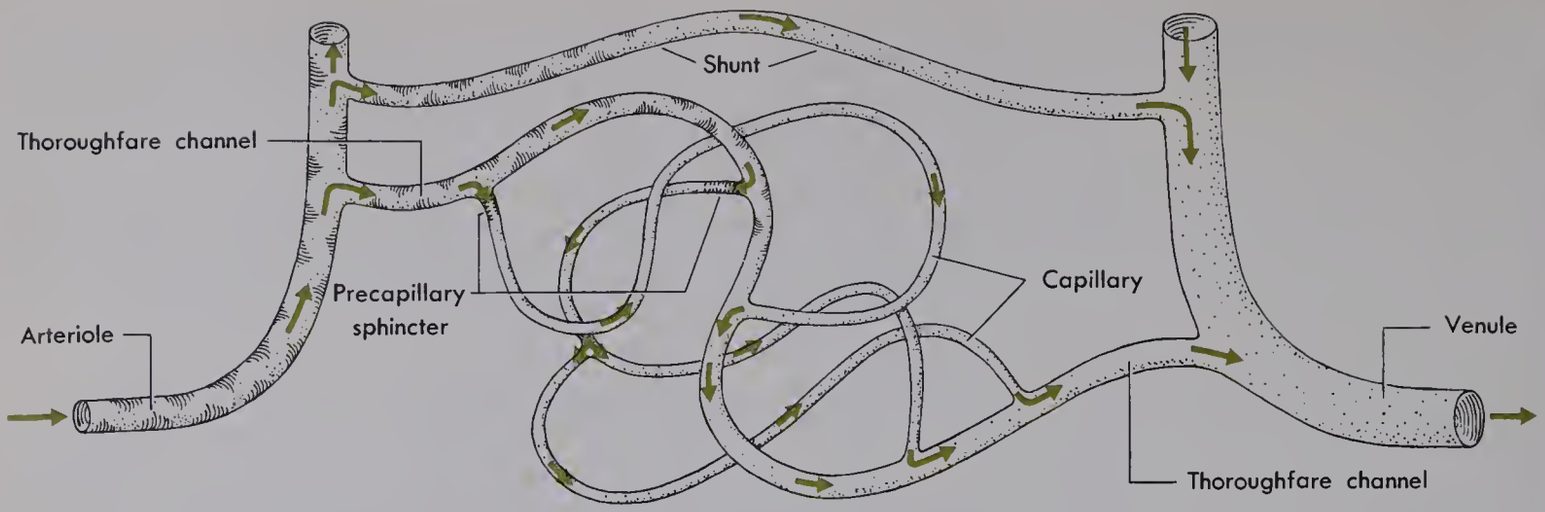


Fig. 13.17 Organization of the capillary bed. Control of blood flow is achieved by changes in the diameter of the arteriole, shunt, thoroughfare channel, and precapillary sphincters. Only *their* walls are muscular.

Shock

The total volume of blood in the body could be contained within the capillary beds alone if they were all open at once. In order to maintain adequate blood pressure and blood flow, it is important that as capillary beds open in one portion of the body, they close in another. Under a few circumstances, this may not occur. Some drugs, pain, even emotional upsets may cause a general dilation of the arterioles and opening of capillary beds throughout the body. Although the volume of blood remains normal, the blood pressure decreases markedly and the victim may go into "shock." If the victim is placed in a horizontal position, the brain and heart will continue to receive sufficient quantities of blood and the chances of recovery are excellent. If maintained in a vertical position, however, the victim will eventually die from lack of oxygen in the brain and heart. This may be the mechanism responsible for death by crucifixion.

Shock can also result from severe bleeding. The heart can pump only as much blood as it receives. If insufficient blood gets back to the heart, the output of the heart (and thus blood pressure) drops. The tissues, especially of the brain and heart, fail to receive adequate supplies of oxygen and glucose. To combat the effects of severe bleeding, arterioles become constricted and capillary beds shut down (except in the brain and heart). This reduces the volume of the system and helps maintain normal blood pressure. In the skin, the flow of blood through the capillary beds may be circumvented completely. Short lengths of vessel, normally closed, make a direct connection between arterioles and venules. These shunts can pass arterial blood directly into the venules and thus hasten its passage back to the heart (Fig. 13.17).

How air-breathing vertebrates conserve oxygen while swimming under water

Air-breathing vertebrates that spend long periods of time under water (such as seals, penguins, turtles, and alligators) employ a similar mechanism to ensure that the oxygen supply of the heart and brain is not seriously diminished. When the animal dives, the blood supply of the rest

of the body is sharply reduced so that what oxygen remains in the blood will be available for those organs that need it most: the brain and the heart. The anatomy of the turtle heart provides an additional mechanism to help with this adjustment. While underwater, a good deal of the blood entering the right side of the ventricle passes through the opening in the septum (see Fig. 13.5) and right back out to the vital parts (e.g. brain) of the body. In so doing, this blood bypasses the lungs, which become less useful anyway as their oxygen content is depleted.

The kidney aids in regulating blood pressure

In mammals, the kidney is responsible for monitoring blood pressure and initiating corrective action if the blood pressure begins to drop. It does this by the release of a proteolytic enzyme, **renin,** which has a very specific activity. Its substrate is a globulin, called angiotensinogen, found in the plasma. Renin splits off a peptide (containing 10 amino acids), called angiotensin I, which can then be acted upon by a peptidase already present in the plasma. The final product is a smaller peptide, containing eight amino acids, which is called angiotensin II. Angiotensin II causes the muscular walls of the arterioles to contract. This closes down capillary beds and by bringing the volume of the functioning blood vessels back into balance with the volume of blood, restores normal blood pressure.

Angiotensin and aldosterone

Angiotensin II has a second, indirect, effect on blood volume and pressure. Perched atop each kidney is an endocrine gland, called the adrenal gland. When angiotensin II reaches the cells of the outer part of the adrenal gland, it causes them to secrete the hormone aldosterone. Aldosterone acts directly on the kidney tubules, enhancing the reabsorption of Na^+ ions. This enhances the reabsorption of water and thus also aids in restoring normal blood volume and pressure.

13.21 CLOTTING OF BLOOD

When blood vessels are cut or ruptured, it is vital that loss of blood from the system be stopped before shock and death follow. Solidification, or clotting, of the blood is able to accomplish this in all but the major vessels. As blood flows out of a damaged vessel, the platelets adhere to the inner surface of the vessel wall. Both they and damaged cells in the area release a fatty material which is activated by certain proteins (clotting "factors") in the blood to form "thromboplastin." In the presence of calcium ions (Ca^{++}) and additional clotting factors in the plasma, thromboplastin catalyzes the conversion of prothrombin (a serum globulin manufactured continuously by the liver) to **thrombin.** Thrombin is an enzyme that catalyzes the conversion of the soluble plasma protein fibrinogen to the insoluble protein **fibrin.** The fibrin gradually forms a mesh in which the blood cells become embedded. Soon a dam (the clot) is constructed which stops loss of blood from the broken vessel.

Twelve substances have been identified that participate in clotting

The various steps in the clotting process are not yet fully understood, and Fig. 13.18 gives no more than an over-all picture. A shortage of any component in the process, e.g. platelets, a plasma clotting factor, or pro-

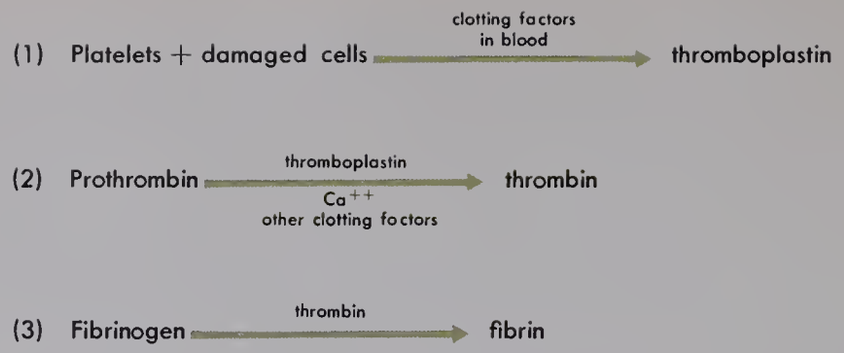


Fig. 13.18 The mechanism of blood clotting.

thrombin, may lead to serious external and/or internal bleeding, even with minor injuries such as a slight cut or the rupture of small blood vessels by a bruise.

Blood is the source of the fluid that bathes all cells

The environment of each of the trillions of cells that make up our bodies is a fluid. This fluid, the interstitial fluid, along with the blood, makes up the ECF of the body. Although interstitial fluid is perhaps a less conspicuous part of our circulatory system than blood, it *is* the internal environment in which our cells live. Interstitial fluid is manufactured from the blood, however, and returns, after bathing the tissues, to the blood. Any change in the physical or chemical properties of the blood immediately results in a corresponding change in the interstitial fluid. In the past few chapters, we have examined several mechanisms that operate to preserve a steady, unvarying chemical composition (and a steady physical state) in the blood. Its temperature, pH, and concentration of glucose, oxygen and carbon dioxide are all held remarkably constant by means of finely adjusted regulatory mechanisms. This constancy of the blood ensures the homeostasis of the interstitial fluid and hence of the true environment, the internal environment, of our bodies. Despite great changes in the external environment, in diet, and in the general activity of the body, our cells continue to operate under conditions that are relatively unvarying and optimum.

In the chapters ahead, we shall examine still other mechanisms by which homeostasis is maintained in the human body. But, before turning to these, let us examine how plants accomplish the transport of materials throughout their bodies.

EXERCISES AND PROBLEMS

1. Why are iron pills prescribed for mild cases of anemia?
2. Citrate ions ($C_6H_5O_7^{\equiv}$) combine with calcium ions (Ca^{++}) to form an insoluble product, $2C_6H_5O_7^{\equiv} + 3Ca^{++} \longrightarrow Ca_3(C_6H_5O_7)_2$. Why do you suppose that a small quantity of sodium or potassium citrate is added to blood collected for storage in blood banks?
3. Trace the path taken by a red blood corpuscle from a capillary bed in your right thumb, to your lungs, and then back to the same capillary bed. Include all vessels, valves, and chambers through which it passes.

4. General edema is characteristic of extreme cases of protein deficiency. Can you account for this?
5. Why is it difficult to see platelets in stained blood slides?
6. In what ways do arteries and veins differ?
7. Which arteries in the human carry deoxygenated blood?
8. How much blood would an adult human need to pump every minute in order to meet his oxygen needs during strenuous exercise if he had no hemoglobin?
9. Summarize the various mechanisms by which our circulatory system adjusts to the demands of strenuous exercise.
10. Trace in detail the path taken by a molecule of O_2 from an RBC into the mitochondrion of a liver cell. Explain what processes are involved at each step.
11. Do the same for a molecule of CO_2 going from a mitochondrion to an RBC.
12. Why are valves needed in veins but not in arteries?
13. Oxygen-transport pigments are found in crustaceans but not in insects. Explain this difference.

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14 THE TRANSPORT OF MATERIALS IN THE VASCULAR PLANTS

Importance

Xylem

Phloem

The Organization of the Root

The Woody Dicot Stem

The Herbaceous Dicot Stem

The Monocot Stem

The Leaf Veins

The Transport of Water and Minerals

Transpiration

Theories of Water Transport

The Transport of Food

14.1 IMPORTANCE

The plant body exists simultaneously in the subterranean world of water and minerals and the aerial world of sunlight and air

Plants are also faced with the problem of transporting materials throughout their bodies. The problem of supply to and from internal tissues is not so acute in plants as it is in animals. As we shall see, the living tissues of a plant are usually quite close to the surface. The real demand for a transport system comes about because of the curious dilemma in which land plants find themselves. A plant cannot carry on photosynthesis without both water and sunlight. It secures its water by sending a root system into the soil. It secures its light by displaying leaves in the air. The greater its success in displaying its leaves above those of competing plants, the farther it has removed them from their supply of water in the soil. An efficient vascular system is needed, therefore, to bridge the gap and transport water quickly from roots to leaves. The vascular system is also needed to ensure that food is transported efficiently from the leaves to the living cells of the stem and roots in order to satisfy their food requirements for metabolism and growth.

The transport of materials in plants is called **translocation.** It takes place in a special system of conducting vessels. These are located in groups known as **vascular bundles** which extend through all the organs of the plant: root, stem, leaf (in the veins), and flower. All the vascular bundles are interconnected so that transport between organs is carried on rapidly and efficiently. Within the vascular bundles, two distinct types of tissue are found, the xylem and the phloem.

14.2 XYLEM

Xylem transports water and minerals up from the roots

The most important part of the xylem tissue of flowering plants is the xylem **vessels** (Fig. 14.1). These are thick-walled tubes which extend vertically through several feet of xylem tissue. They range in diameter from only 20 μm to as much as 700 μm (0.7 mm), depending upon their location and the species in which they are present. The walls of the xylem vessels are thickened with secondary deposits of cellulose and are usually further strengthened by impregnation with a cementing material, lignin. The secondary walls of the xylem are not deposited evenly, but in spirals, rings, etc. The walls of xylem vessels may also be perforated by pits.

The xylem vessels arise from individual, cylindrical cells usually oriented end to end. At maturity, the end walls of these cells dissolve away and the cytoplasmic contents die. The result is the xylem vessel, a continuous nonliving duct. The xylem vessels function in the upward transport of water and minerals.

Also present in the xylem tissue are xylem **tracheids.** These are individual cells about 30 μm in diameter and several millimeters in length. In cross section, they can be distinguished from the xylem vessels by their angular walls and, in many cases, smaller size. They taper at each end and the tapered ends of one cell overlap the tapered ends of the adjacent cells. Like xylem vessels, they possess thick, lignified cell walls and, at maturity,

no cytoplasm. Their walls are perforated so that water and dissolved minerals can, and do, flow readily from one tracheid to the next. Like the xylem vessels, they transport water and minerals upwards through the vascular bundle. In ferns and conifers, the xylem tracheids are the only water transport ducts. Vessels are not present.

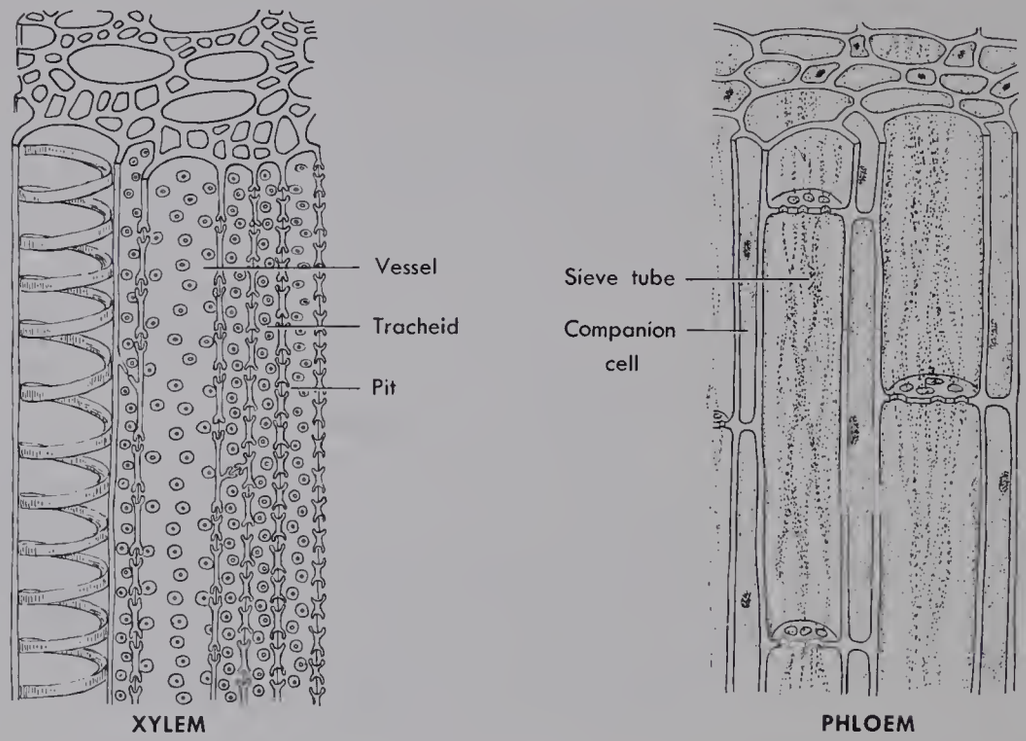


Fig. 14.1 Vascular tissues. Xylem vessels and tracheids (left) contain no cytoplasm at maturity.

14.3 PHLOEM

The phloem transports food from the leaves to the rest of the plant

The major conducting vessels of the phloem are the sieve tubes. These consist of cylindrical cells (about 25 μm in diameter and 100 μm long) oriented end to end. The end walls of mature sieve-tube cells are perforated, permitting strands of cytoplasm to extend between adjacent cells. The appearance of these end walls under the microscope has caused them to be called sieve plates (Fig. 14.1). The side walls of the sieve-tube cells are also perforated. Fine strands of cytoplasm, the plasmodesmata, extend through these pores, connecting sieve-tube cells that lie side by side. Like the xylem vessels and tracheids, the sieve tubes form continuous ducts extending from the bottom to the top of the plant.

The sieve tubes differ from the xylem vessels and tracheids in that the constituent cells do not lose their cytoplasm at maturity. They do, however, lose their nucleus. In many plants, the cells of the sieve tubes lie adjacent to companion cells (Fig. 14.1) which do retain their nucleus at maturity and may possibly exert some control over the activities of the cells of the sieve tube. The sieve tubes transport foods and plant hormones both upwards and downwards in the plant.

The xylem and phloem make up the vascular bundles of the plant. The organization of these vascular bundles is different in the root, stem, and

veins of the leaf. Furthermore, the arrangement of vascular bundles within a single organ varies considerably from species to species. In considering the internal organization of the root, stem, and the leaf we shall necessarily restrict ourselves to just a few representative types.

Monocots and dicots

There are two major subdivisions of flowering plants: the dicotyledons (**dicots,** for short) and the monocotyledons (**monocots**). These names arise because of the occurrence of two food storage organs, or cotyledons, in the seeds of the first group and only one in the seeds of the second. Dicots include an enormous variety of plants. Buttercups, cabbages, legumes, cacti, tomatoes, elms, oaks, and maples are just a few of the many kinds of dicots. The monocots include lilies, palms, orchids, tulips, onions, and all the grasses. Grasses, in turn, include corn, wheat, rice, and all the other cereal grains upon which we depend so heavily for food.

Monocots and dicots differ in a number of ways other than in their seed structure. One of these is in the pattern of organization of the vascular tissues. Other differences will be touched on in Chapter 35.

14.4 THE ORGANIZATION OF THE ROOT

The root tip

Figure 14.2 shows the structure of a typical young dicot root. At the tip of the root is a meristem that produces the cells from which the first (primary) root structures will develop. Mitosis in this meristem increases the length of the root. Because of the frequency with which mitosis occurs in this "embryonic" region, root tips are frequently used to demonstrate and study this type of cell division. The meristem is protected from abrasion and damage in the soil by the root cap.

As soon as cells are manufactured by the meristem, they undergo a period of elongation. The region of the root in which this occurs is known as the region of elongation.

Once the root cells are fully elongated, they begin the process of **differentiation.** Differentiation involves the development of specialized structures. Cells at the surface of the root differentiate to form epidermal cells. Most of these develop long extensions of the wall, the **root hairs.** These greatly increase the surface area of the root and are the main point of entrance for water.

Within the epidermis develops a ring (as viewed in cross section) of parenchyma cells, the **cortex.** The cortex serves as a food storage area. Its inner surface is bounded by a single layer of cells, the **endodermis.** Within the endodermis is the central cylinder containing the vascular bundles. Surrounding the central cylinder is the **pericycle,** from which branches of the root (secondary roots) arise. Within the pericycle of the young root are xylem tissue, phloem tissue, and parenchyma or pith. The xylem tissue is arranged in bundles in a radial or spokelike fashion. The phloem tissue alternates with the xylem tissue (Fig. 14.2).

In the older root, another meristem, the cambium, develops between the xylem and phloem. Mitosis in the cambium produces new (secondary) xylem to the inside and new (secondary) phloem to the outside (Fig. 14.2).

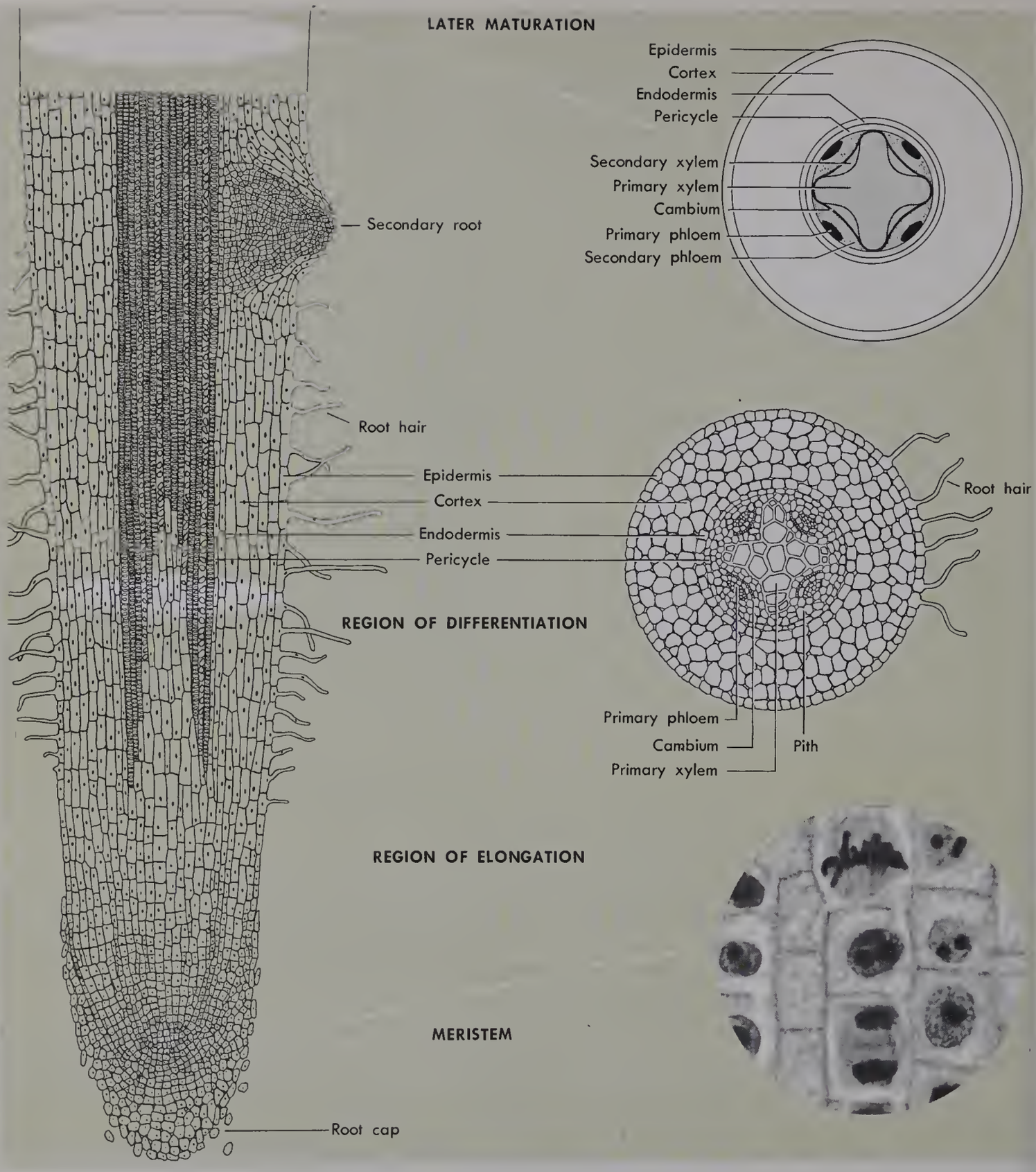


Fig. 14.2 Organization of a young dicot root in longitudinal section (left) and cross sections (right). The longitudinal section has been shortened to enable each stage in root development to be shown. (Photo courtesy of General Biological Supply House, Inc.)

The vascular tissues of the root connect with those of the stem

The vascular bundles of the stem are simply extensions of those in the roots. They are, however, arranged somewhat differently. Furthermore, the arrangement of bundles in the monocot stem is markedly different from that in the dicot stem. (The organization of tissues in monocot roots and dicot roots is essentially alike.) Even among the dicots, the arrangement of tissues in those that develop woody stems (perennials) is somewhat different from those whose stems are herbaceous (annuals).

14.5 THE WOODY DICOT STEM

A cross section of a young twig of the basswood or American linden (*Tilia*) provides a good example of the organization of tissues in the typical woody dicot (Fig. 14.3). The twig is made up of three distinct regions, the bark, the wood, and the pith. The outer surface of the bark region is protected by layers of dead cork cells impregnated with suberin. Suberin is a waxy material which markedly cuts down water loss from the stem. A great many openings, the lenticels, are present in the cork and it is by means of these openings that oxygen and carbon dioxide can be exchanged between the stem tissues and the air. Beneath the cork are layers of parenchyma cells which make up the cortex. These cells store food in the stem just as they do in the root. In the very young stem (before cork has developed) they may have chloroplasts and carry on photosynthesis. In the

Cork

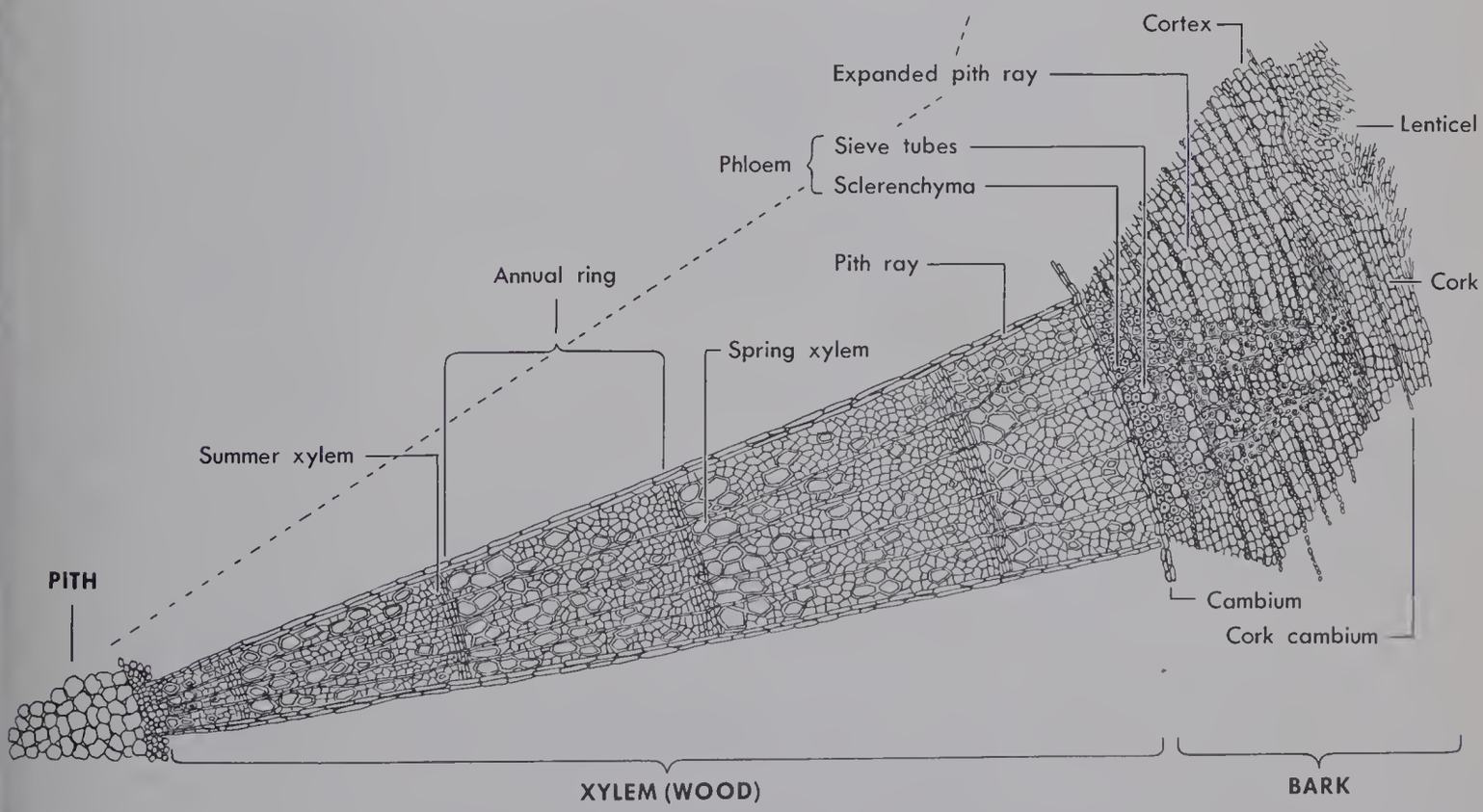


Fig. 14.3 The woody dicot stem. A cross section showing a single vascular bundle of *Tilia,* the American linden (basswood). How old was this twig when it was cut?

older stem a meristem develops between the cortex and the cork. Mitosis in this meristem (the cork cambium) replaces the cork cells that are lost by weathering.

In woody dicots, the phloem is confined to the bark

The inner portion of the bark is marked by alternating areas of phloem tissue and parenchyma. The parenchyma makes up the terminal portion of the horizontal rays that run between the pith and the bark. These rays carry on lateral transport of materials between the two regions. The enlarged portion in the bark also serves as a food storage area. The phloem tissue consists of bundles of sieve tubes surrounded by and supported by sclerenchyma cells.

The inner boundary of the bark region is marked by a meristem, the cambium. As a result of its activity, new phloem is continually produced in the bark region during the growing season. The cambium also produces new xylem towards the interior.

Wood is xylem

Xylem makes up the wood region. The xylem vessels produced during the spring, when water is plentiful, have larger diameters than those produced later in the summer. During the dormant season, no xylem whatsoever is formed. The visual contrast between the summer xylem of one year and the spring xylem of the next year permits the easy counting of annual rings. The entire band of xylem formed in one growing season makes up one annual ring. How old was the basswood twig shown in Fig. 14.3 when it was cut?

The xylem serves a dual function: support and transport. In older stems, only the most recent rings of xylem participate in the transport of materials. These make up the **sapwood.** The inner rings of xylem cease to function in transport, but provide ever-increasing support for the weight of the tree above. This portion of xylem is called the **heartwood.**

The innermost portion of the young basswood twig is the pith. It contains parenchyma cells and serves as a food storage area. In the older woody stem, the pith disappears.

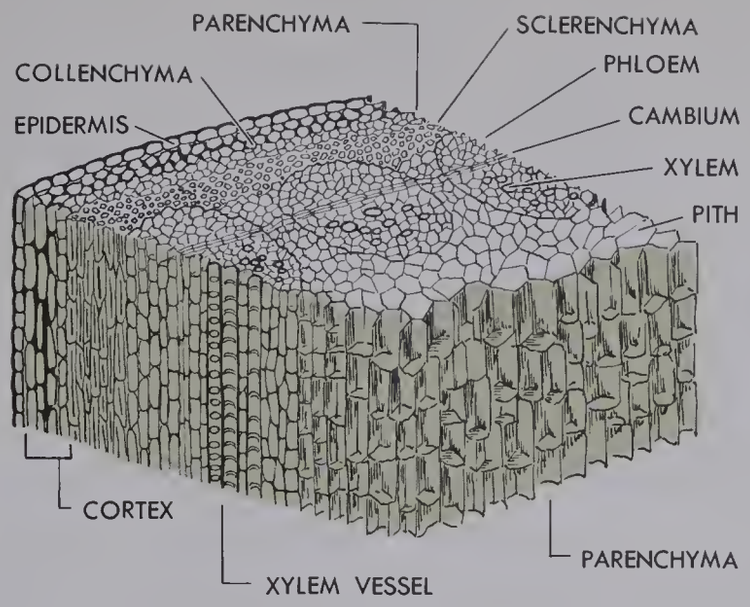
14.6 THE HERBACEOUS DICOT STEM

Herbaceous stems carry on photosynthesis

The basic arrangement of the herbaceous dicot stem (Fig. 14.4) is similar to that of the woody dicot, although the surface of the stem is protected only by a layer of epidermis rather than by cork. Beneath the epidermal cells are cortex cells. These often possess chlorophyll and carry on photosynthesis. The interior of the stem is packed with parenchyma cells, the pith. The outer portions of the vascular bundles contain phloem tissue, just as in the woody dicot. On the inner side, the phloem is separated from the xylem by a cambium. Although the cambium does produce additional xylem and phloem during the growing season, no annual rings are formed.

As its name implies, the herbaceous dicot stem lacks the strength and rigidity of the woody dicot stem. This is because there are no rings of woody xylem present. The stem does receive some mechanical support, however, from masses of sclerenchyma located between the phloem and the cortex.

Fig. 14.4 Anatomy of a typical herbaceous dicot stem. Why are there no annual rings?



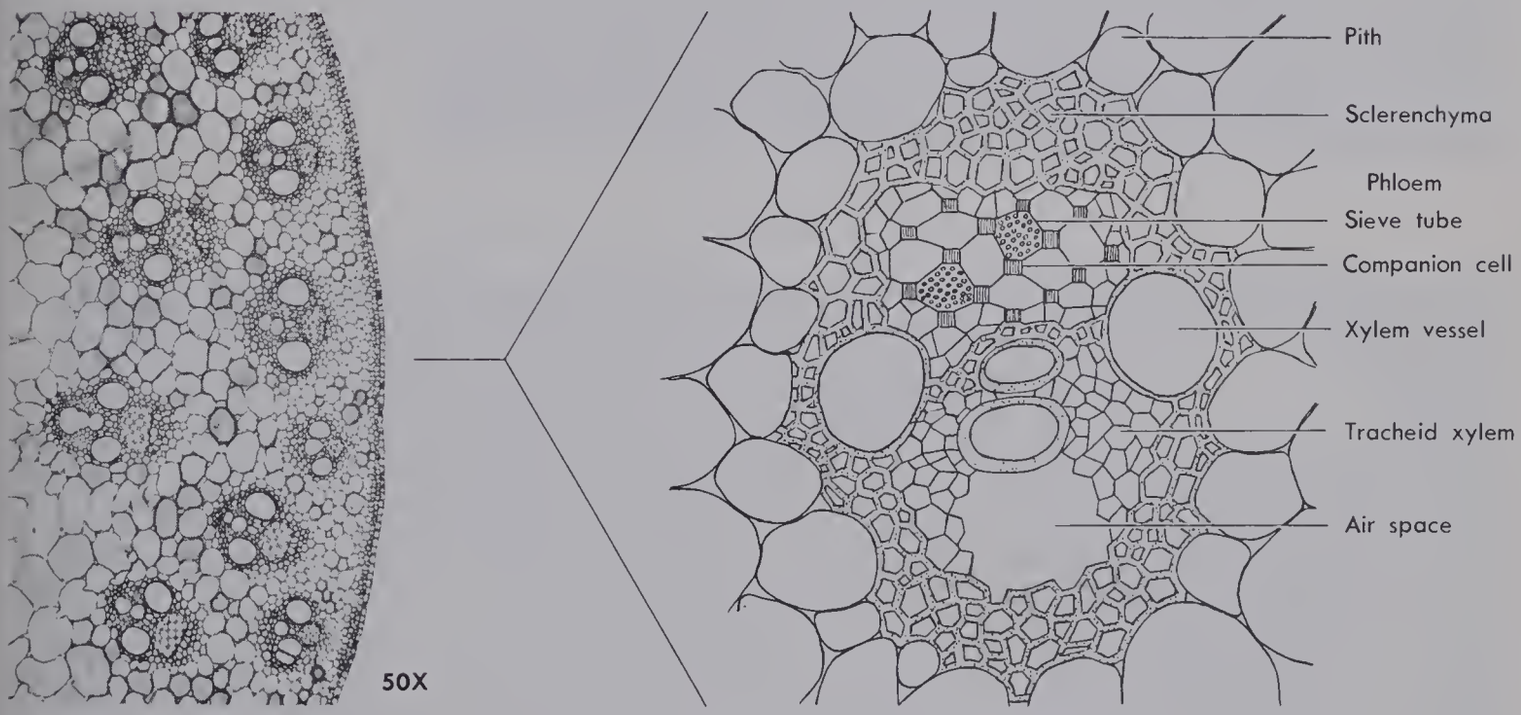


Fig. 14.5 Organization of a typical monocot stem. Left: a cross section of a portion of a corn stem (*Zea mays*). (Courtesy of General Biological Supply House, Inc.) Right: detail of a single vascular bundle.

14.7 THE MONOCOT STEM

Most monocots are herbaceous

The organization of the monocot stem is quite different from that of the dicot. A cross section of a corn stem (*Zea mays*) (Fig. 14.5) shows these differences nicely. The corn stem consists of an external rind and an interior filled with pith. The rind provides most of the mechanical support for the stem. Scattered pretty much at random through the pith are the vascular bundles. In cross section, they remind one of a caricature of a human

face. Each is surrounded by a layer of sclerenchyma cells that provides mechanical support for the cells within. The phloem is located in the forehead region. Careful examination reveals companion cells located between the sieve tubes. Four xylem vessels are present, making up the two "eyes," the "nose," and "mouth." Much of the bottom xylem vessel is surrounded by air in the air space. Xylem tracheids are present in the "cheek" areas.

14.8 THE LEAF VEINS

The vascular bundles of the leaves are direct extensions of the vascular bundles of the stem. They pass from the stem into the leaf stalk and then into the various veins of the leaf. These branch into ever-finer veins (Fig. 14.6). Probably no cell in the spongy layer of the leaf is more than two cells away from the end of a vein. The xylem and phloem of the vein (Fig. 9.8) are often surrounded by layers of sclerenchyma cells which impart strength to the vein. The veins, in turn, provide a rigid framework to support the remaining soft tissues of the leaf blade.

THE TRANSPORT OF WATER AND MINERALS

14.9 THE PATHWAY

Root hairs

Water enters the plant through the root hairs. These extensions of the epidermal cells have sticky walls and adhere tightly to soil particles. This places them in direct contact with the film of water that also adheres to the soil particles of all but "bone-dry" soils. Root hairs are found only at the tips of the roots, but there may be a great many of them. Careful measurements have been made of the root system of the grass, rye. After four months of growth, a single plant was found to have 387 miles of roots. There were approximately 13 million root tips possessing an estimated total of 14 billion root hairs (Fig. 14.7). Once within the epidermal cells, water passes through and between the cells of the cortex. It enters the central cylinder by passing through the endodermis and pericycle. In many roots, the endodermis contains specialized cells, the passage cells, which provide an easy pathway for the movement of water into the central cylinder. In the young root, water then enters the xylem directly. In older roots, it may have to pass first through a band of phloem and cambium. It does this by travelling through horizontally elongated cells, the xylem rays.

Minerals are actively transported into the root

One might suppose that minerals enter and move through the plant simply dissolved in water. While water and minerals do share the same final pathway, the uptake of minerals by the root has several special features. Even when no water is being absorbed by the roots, minerals

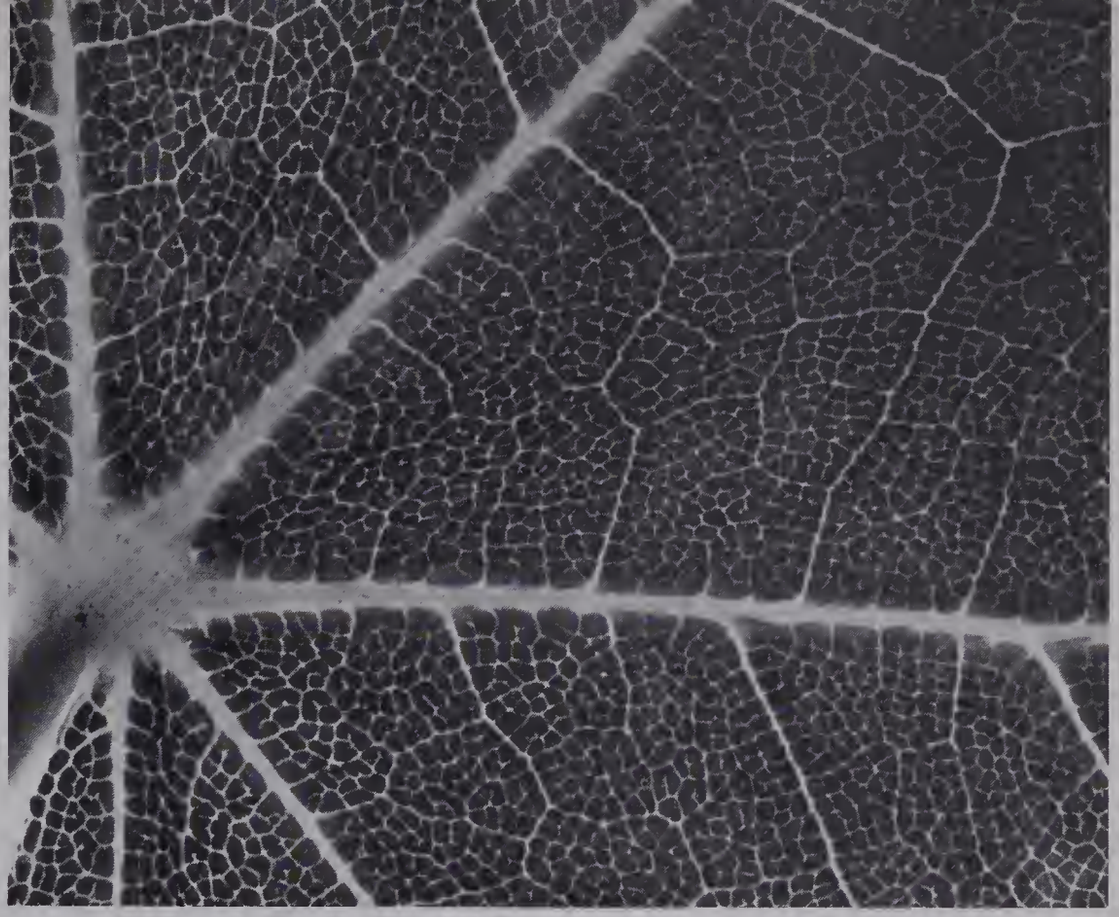


Fig. 14.6 Veins in a maple leaf.

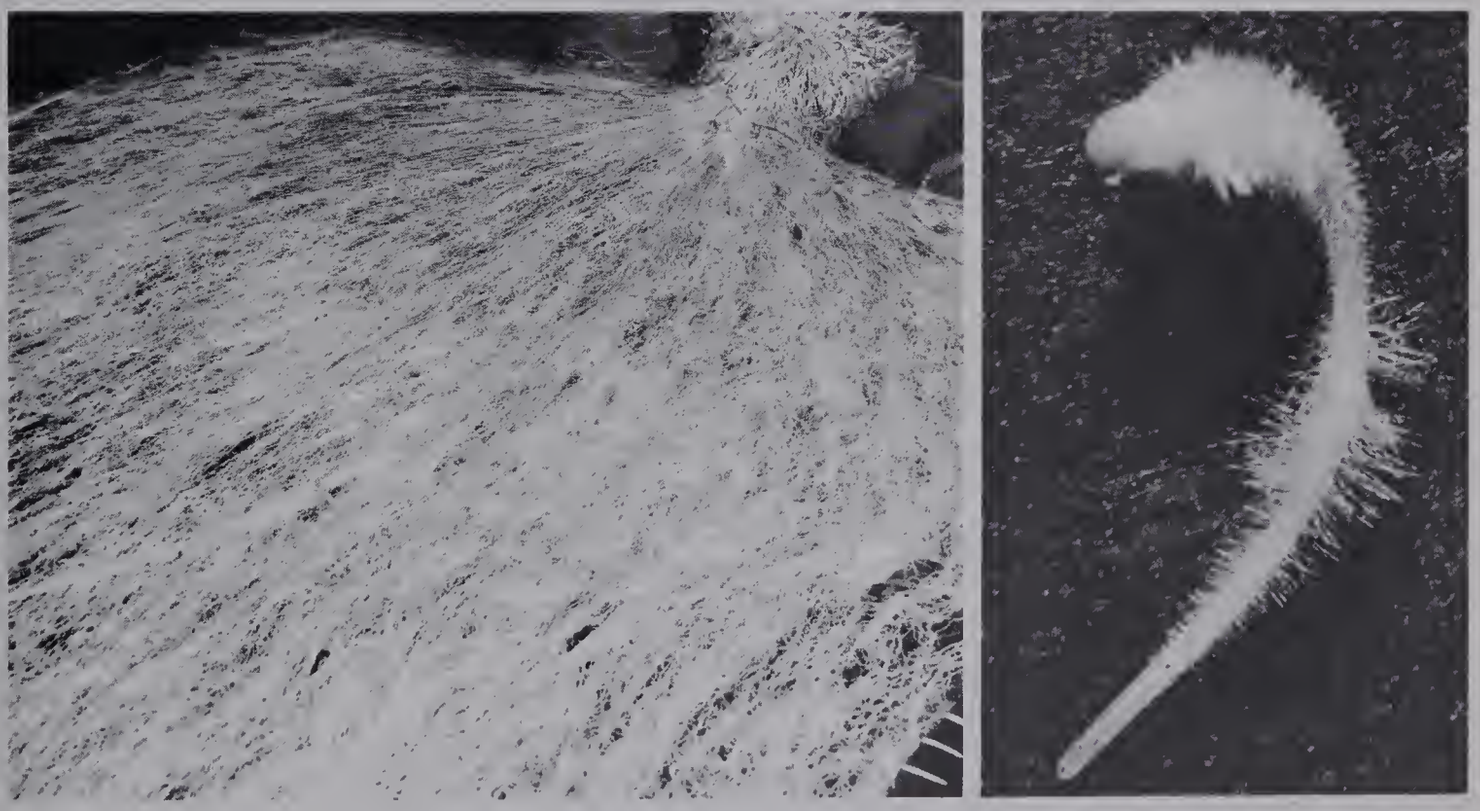


Fig. 14.7 Absorptive area of roots. Left: after two years of growth, a single plant of crested wheat grass had produced 319 miles of roots. (Courtesy of "Science Service.") Right: the surface area for water absorption is increased further by the root hairs at the tip of each root (seen on the primary root of a germinating radish.)

enter freely. Furthermore, they can enter against a concentration gradient, i.e. from a region of lower concentration (the soil) to a region of higher concentration (the cells of the root). Hence, active transport must be at work and, indeed, anything which interferes with active transport processes in general also blocks mineral uptake.

Plants absorb soil nutrients in inorganic form

Perhaps it should be emphasized that all the elements needed by the plant are taken up in inorganic form, hence as "minerals." Nitrogen enters as nitrate (NO_3^-) or ammonium ions (NH_4^+), phosphorus as $PO_4^{\equiv}$, potassium as K^+, calcium as Ca^{++}, etc. When one hears the virtues of organic fertilizer being extolled, it should be remembered that such material meets no nutritional needs of the plant until it has been degraded to inorganic form. Organic matter does play an important role in the formation of good soil texture, but only to the extent that it can yield inorganic ions can it meet the nutritional needs of plants.

Once within the epidermal cells of the root, inorganic ions pass inward from cell to cell, probably by way of plasmodesmata (see Section 14.3). The final step from the cytoplasm of the pericycle cells to the xylem is probably accomplished once again by active transport.

Once in the xylem, water and the minerals that have been deposited in it move upward in the vessels and tracheids. These run up through the root and the stem. At any level the water can leave the xylem and pass laterally to supply the needs of other tissues. At the leaves the xylem passes into the leaf stalk (the petiole) and then into the veins of the leaf. A glance at the scars left on woody stems after the leaves have dropped in the autumn will reveal the severed ends of the vascular bundles that had supplied the leaves (Fig. 23.3).

At the ends of the veins, water leaves the xylem and enters the spongy layer and palisade layer cells of the leaf. Here the water may be used in photosynthesis or may be evaporated from the leaf in the process of transpiration.

Several bits of evidence lead to the conclusion that the upward transport of water takes place in the xylem. For example, if a ring of bark is carefully removed from the trunk of a tree, thus removing all the phloem, the upward passage of water is not interrupted. Such an operation is called girdling. Young fruit trees are all too frequently girdled during the winter by hungry field mice feeding upon the bark. With the arrival of spring, the tree develops leaves normally, suggesting that the upward movement of water and minerals has not been interfered with. Unless a special graft (Fig. 14.8) is made, however, the tree will ultimately die. (Why?) Additional evidence is obtained when the severed top of a plant is placed in water containing a dye. The water is drawn up into the stem for some period of time; microscopic examination of the vascular tissue shows that the dye is localized in the xylem vessels.

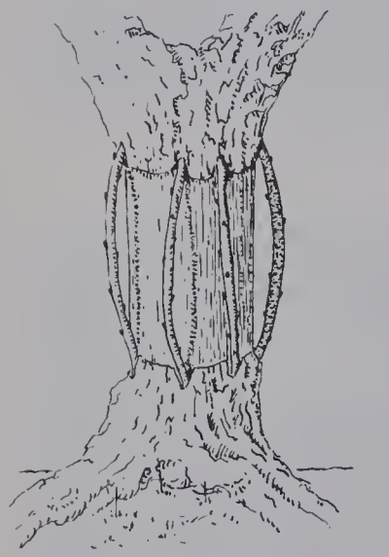


Fig. 14.8 A bridge graft. Without it, the roots would die for lack of food.

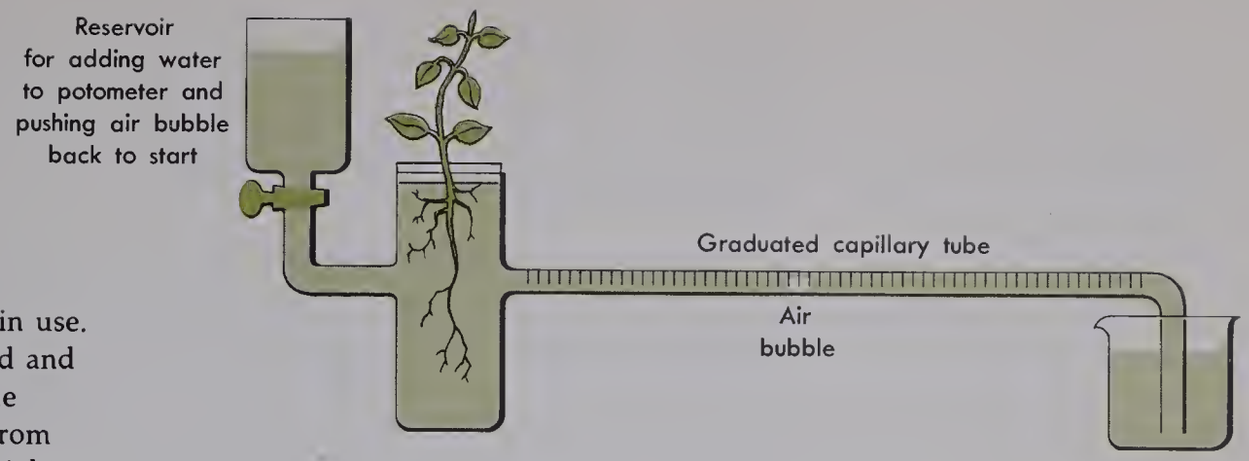


Fig. 14.9 Potometer in use. As water is transpired and otherwise used by the plant, it is replaced from the reservoir on the right. This pushes the air bubble to the left and permits the amount of water used to be measured accurately.

14.10 MAGNITUDE OF FLOW: TRANSPIRATION

The forces responsible for the movement of water through the xylem are not yet fully understood. Any theory to explain the upward movement of water in the xylem must take into account the large volume of water transported and the speed with which the water travels. Herbaceous plants may absorb a volume of water each day equal to several times the volume of the plant itself. Water that contains a marker (for example a radioactive isotope) has been shown to move up the stem as much as 75 cm each minute.

Transpiration is the evaporation of water from the exposed surfaces of the plant

Only 1–2% of all this water is used in photosynthesis or in other metabolic activities of the leaf cells. The remainder evaporates from the leaf in the process of **transpiration.** In Chapter 12, you learned that water evaporates into the air spaces of the spongy layer. When the stomata open, the water vapor diffuses out of the leaf. If the leaf is to continue to function properly, fresh supplies of water must be brought to the leaf to replace that lost in transpiration.

Measuring the rate of transpiration

Figure 14.9 illustrates a device with which it is possible to approximate the amount of water vapor given off in transpiration. As water is absorbed by the roots of the plant, it is replaced by water drawn through the graduated tube from the reservoir on the right. A small bubble introduced into the system serves to mark the volume consumed. Although this device, a potometer, measures the total water use of the plant, 98–99% of this is accounted for by transpiration.

14.11 FACTORS AFFECTING THE RATE OF TRANSPIRATION

Using a potometer, it is possible to study the effect of various environmental factors upon the rate of transpiration.

1. Light. Plants transpire much more rapidly when exposed to light than in the dark. This is chiefly because light stimulates the opening of the stomata (see Section 12.4) and thus greatly increases the transfer of moisture-laden air from the air spaces of the spongy layer to the outside. Light also speeds up transpiration by warming the leaf.

Roughly 99% of the water absorbed by plants is lost in transpiration

2. Temperature. Plants transpire more rapidly at higher temperatures than at low. At 30°C a leaf may transpire three times as fast as it does at 20°C. This is because water evaporates more rapidly as its temperature increases and, in this case, thus increases the humidity of the air in the air space with respect to that outside.

3. Humidity. Rate of transpiration is also affected by the relative humidity of the air surrounding the plant. The rate of diffusion of any substance is decreased as the difference in concentration of the substance in the two regions decreases. The reverse is also true. Therefore diffusion of water from the moisture-laden air spaces of the leaf to the outside goes on rather slowly when the surrounding air is quite humid. When the surrounding air is dry, diffusion proceeds much more rapidly.

4. Wind. The presence of gentle air currents also increases the rate of transpiration. When none are present, the air immediately surrounding a transpiring leaf becomes increasingly humid. As it does so, it causes a decrease in the rate of transpiration for the reason just mentioned. When a breeze is present, however, the humid air is carried away and replaced by fresh, drier air.

5. Soil Water. A plant cannot continue to transpire rapidly if its moisture loss is not made up by absorption of fresh supplies of water from the soil. When absorption of water by the roots fails to keep up with the rate of transpiration, loss of turgor occurs, and the stomata close. This immediately reduces the rate of transpiration greatly. Often the loss of turgor may extend to other plant parts and wilting occurs.

Under optimum conditions, the loss of water through transpiration is truly impressive. It has been estimated that over the course of the growing season an acre of corn plants may transpire 400,000 gallons of water. In liquid form this would be enough to cover the field with a lake 15 inches deep. The quantity of water transpired per acre by the trees of a mature deciduous forest is probably even greater.

Substantial forces are required to move water to the top of tall trees

Not only must a theory of water transport in the xylem account for the large quantities of water involved, but it must also account for the great height to which the water may be transported. The sequoias and Douglas firs of the Pacific coast regularly attain heights of 300 feet or more. The weight of a column of water this high exerts a pressure of almost 140 lb/in^2. Taking into account the speed at which water moves up the xylem and the resistance to this flow created by the walls of the tracheids, it seems likely that a push (or pull) of 300–400 lb/in^2 is called for.

Any theory to explain the upward transport of water must be based on purely physical principles. Remember that the xylem vessels and tracheids are quite lifeless. This was shown dramatically by a German botanist who sawed down a 70-foot oak tree and placed the base of the trunk in a barrel of picric acid solution. The solution was drawn up the trunk, killing nearby tissues as it went. Three days later water, to which a dye had been added, was still moving successfully to the top of the tree.

THEORIES OF WATER TRANSPORT

Two theories that have been proposed to explain the upward transport of water in the xylem deserve mention.

14.12 ROOT PRESSURE

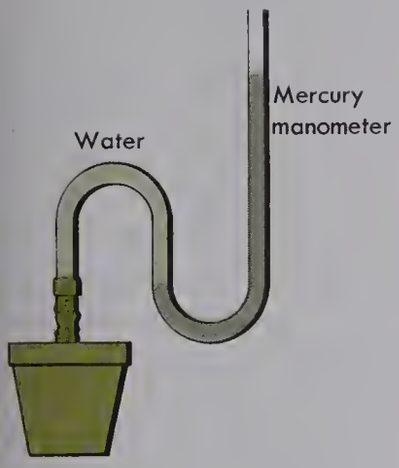


Fig. 14.10 Measuring root pressure in the tomato.

How root pressure is created

When a tomato plant is carefully severed close to the base of the stem, a fluid—sap—oozes from the stump. If a pressure-measuring device is connected to the stump, it is found that the sap is being pushed out under considerable pressure (Fig. 14.10). This is known as root pressure. Although the experiment is not an easy one to perform, careful workers have reported root pressures in the tomato as high as 130 lb/in^2. While this pressure would not lift sap to the top of a sequoia, it would easily lift it to the top of the tomato plant and even to the top of most trees.

The origin of root pressure is thought to lie in the difference in water concentration of the soil water and the sap in the xylem ducts. Xylem sap may be hypertonic to soil water because of the presence of dissolved sugars, or salts that have been actively transported into the central cylinder. This is particularly apt to be true in woody perennials like the sugar maple when, in very early spring, they hydrolyze the starches stored in their roots into sugar. At such a time, it is theorized, water would pass by osmosis from the root hairs, through the cortex and endodermis, to the xylem ducts. The inflow of water into the sap-filled xylem ducts would create a pressure, which could then be relieved by movement of the sap up the ducts. This is bascially the same mechanism by which the molasses solution rose in the osmometer described in Section 6.4.

While root pressure is now a demonstrated fact, it seems unlikely that it can account completely for the upward transport of water in the xylem. Although substantial root pressures have been measured in the tomato, practically no other plants have been found to develop root pressures greater than 30 lb/in^2. Furthermore, it has been impossible to demonstrate any root pressure at all in many plants.

The volume of fluid transported by root pressure is also not adequate to account for the measured movement of water in the xylem. Furthermore, those plants whose roots produce a reasonably good flow of sap are apt to have the lowest pressure, and vice versa.

There is still another difficulty with the root pressure theory of water transport. Even in those plants that show root pressures, the highest values occur in the spring when the sap is strongly hypertonic to soil water but the rate of transpiration is low. In summer when transpiration is proceeding rapidly, and hence water is moving through the xylem rapidly, often no root pressure can be detected at all.

Thus, while root pressure may play a significant role in water transport in certain species (recent studies indicate that it may be the most important factor in the coconut palm) and at certain times, we must look for an alternative mechanism.

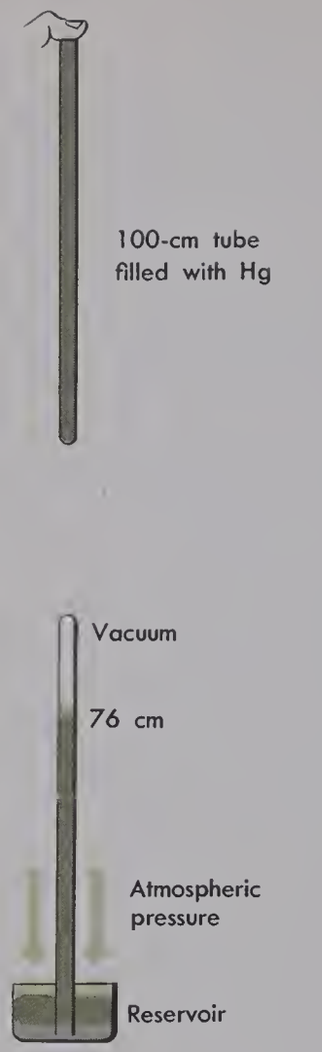


Fig. 14.11 Establishment of Torricellian barometer and effect of placing the barometer in a vacuum chamber.

14.13 THE DIXON-JOLY THEORY

In 1895, the Irish plant physiologists H. H. Dixon and J. Joly proposed another theory to explain the rise of water in plants. They thought that water, instead of being pushed up by root pressure from below, was pulled up by tension (negative pressure) from above. As we have seen, water is continually being used at the leaves. Transpiration accounts for most of this, but photosynthesis and other biochemical activities in the cell account for some consumption of water, too. According to the Dixon-Joly theory, the use of water in the leaf exerts a pull on the water in the xylem ducts and draws more water into the leaf. The pull on the water in the xylem vessels is, in turn, transmitted to the roots and pulls water from the soil into the xylem ducts of the root.

Anyone who has studied physics knows that even the finest vacuum pump can only pull water up to a height of 32 feet. This is because a column of water that high exerts a pressure (about 15 lb/in^2) just counterbalanced by the pressure of our atmosphere (Fig. 14.11). How, then, can water be drawn up 300 or more feet under tensions of 300–400 lb/in^2?

The answer to this question lies in the property of water molecules to cling to each other. This property is known as **cohesion.** When water is confined to tubes of very small bore, the force of cohesion between the water molecules imparts great strength to the column of water. It has been found experimentally that tensions as great as 3000 lb/in^2 are necessary to break such a column of water. This compares very favorably with the strength of steel wires of the same diameter. According to the Dixon-Joly theory, the great cohesive strength of the tiny filaments of water in the xylem ducts, coupled with the property of water molecules to *adhere* to the walls of the ducts, enables the filament of water to be pulled to the top of the tallest trees without breaking or pulling away from the walls of the ducts. Water use at the leaves, chiefly in transpiration, creates the tension which is then transmitted back down the xylem all the way to the root hairs. (The Dixon-Joly theory has often been called the transpiration pull-cohesion theory.) In a real sense, the tiny filaments of water in the xylem possess the physical properties of solid wires.

14.14 EVIDENCE FOR THE THEORY

What evidence is there to support this theory? Figure 14.12 illustrates an experimental model which shows that it is physically possible to raise water by such a mechanism. Evaporation of water at the porous clay cup creates a tension which is transmitted to the water and mercury in the glass tube. When great care is taken to see that no dissolved air is present in the water, it has been possible to draw the mercury column as high as 226 cm. This is equivalent to raising a column of water almost 100 feet into the air, and is three times greater than the height that atmospheric pressure working against a perfect vacuum could attain.

The model shows only that the phenomenon is possible. It does not prove that it takes place. When a cedar twig is substituted for the porous

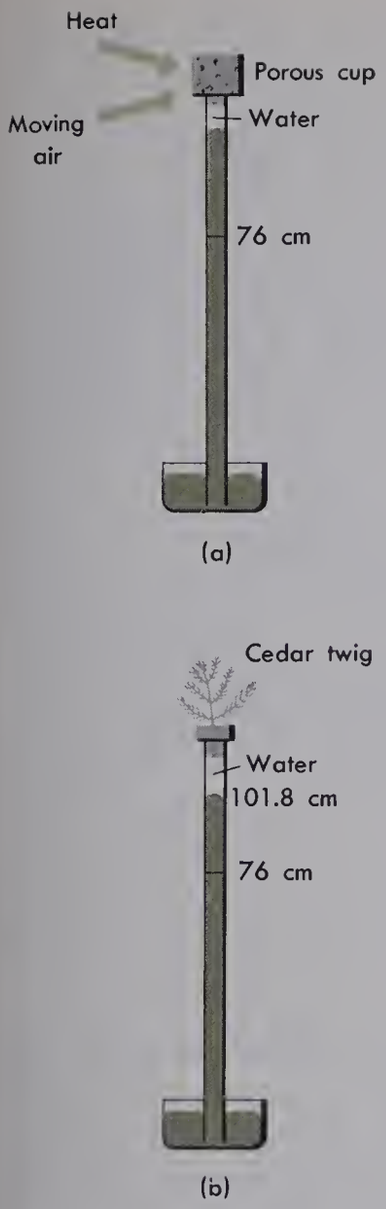


Fig. 14.12 Rise of mercury column in excess of 760 mm as a result of evaporation from (a) a porous cup and (b) a cedar twig.

cup, however, the mercury is again lifted higher than the height (about 76 cm) that could be attained with a perfect vacuum.

Accepting the theory that the water in the xylem ducts is indeed under tension rather than pressure, it is possible to deduce certain facts which must follow from this. By checking to see if these predicted facts are indeed so, one is able to test the validity of the theory. If the water in a xylem vessel is under tension, we would expect the column to snap apart if air is introduced into the vessel by puncturing it. This has been found to be so.

Furthermore, if the water in all the xylem ducts is under great tension, there should be a resulting inward pull (because of adhesion) on the walls of the ducts. The inward pull on the walls of all the ducts in the band of sapwood in an actively transpiring tree should, in turn, result in a decrease in the diameter of the tree. This deduction can be *tested* by continuously measuring, with suitable equipment, the diameter of tree trunks. Figure 14.13 shows the results that the American plant physiologist D. T. MacDougal obtained with the trunk of a Monterey pine tree. The diameter of the tree did indeed fluctuate, with its minimum diameter being reached just after midday, the time of greatest transpiration.

In 1960 a team of plant physiologists working in the jungles of Northeastern Australia carried out experiments on the rattan vine which lent additional support to the Dixon-Joly theory. The rattan vine climbs upon the trees of the jungle. In order to get its foliage into the sun, it may have to climb as high as 150 feet into the air. When the base of the vine is severed while immersed in a basin of water, water continues to be taken up by the vine. Obviously, root pressure is playing no part in the process, because the vine has been detached from its root system. A rattan vine less than 1 inch in diameter will "drink" water indefinitely at a rate of about 12 ml each minute. If forced to take up water from a sealed container, the vine does so without any decrease in rate even though a high vacuum is quickly produced in the container. In fact, the vacuum becomes so great that the remaining water begins to boil spontaneously. (The boiling temperature of water decreases as the air pressure over the water decreases.)

The Dixon-Joly theory provides an explanation for the ability of certain vascular plants to live in salt water. The roots of the coastal mangrove grow immersed in salt water, a solution markedly hypertonic to the cytoplasm of their cells. On the basis of respective water concentrations, we could expect water to leave the cells of the mangrove with resulting plasmolysis. Remember how the cells of *Anacharis* become plasmolyzed in

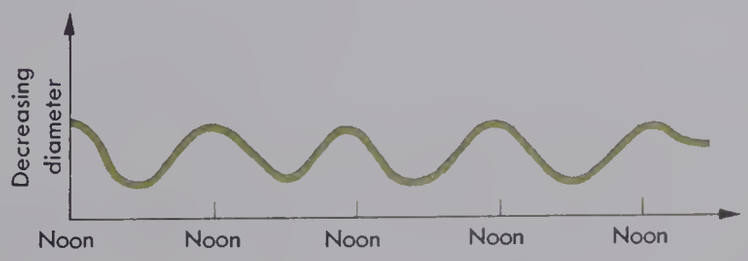


Fig. 14.13 Diurnal (daily) variation in the diameter of the trunk of a Monterey pine. The diameter of the trunk reaches its minimum when the rate of transpiration reaches its maximum.

salt water (see Fig. 6.11). However, remarkably high tensions have been measured in the vascular system of coastal mangroves. These tensions (on the order of 500–800 lb/in^2) are so great that they can pull water molecules into the plant against the osmotic gradient. By this mechanism, mangroves literally desalt sea water to satisfy their water requirements.

On the basis of all the evidence, the Dixon-Joly theory seems to provide a satisfactory explanation of the rise of liquids in plants. Though several questions remain to be answered, most plant physiologists feel that the theory has been well tested and best describes the mechanism by which most, if not all, plants transport water and dissolved minerals from the soil to the leaves.

THE TRANSPORT OF FOOD

14.15 THE PATHWAY

Foods and other substances (e.g. hormones) manufactured by the plant are also transported in the vascular bundles. Sugars (usually sucrose), amino acids, and other organic molecules that have been manufactured in the leaves enter the cells of the phloem. This entry is probably facilitated by the plasmodesmata which connect the photosynthesizing cells of the leaf with the cells of the phloem.

The organic molecules synthesized in the leaf are transported to other parts of the plant by way of the phloem

Once within the phloem, the sugar and other organic molecules may be transported either up or down to any region of the plant. In the case of sugar destined to be stored in the roots, it passes in solution through the phloem of the stem and root. Once within the root, the sugar then passes out of the phloem, through the pericycle and endodermis and into the cells of the cortex. Here it can be converted into starch and stored as a food reserve.

Demonstrations that food is transported in the phloem

That food transport occurs in the phloem is suggested by girdling experiments. In summer, when a tree is girdled so that the phloem is removed but the xylem remains intact, the tree continues to live. There is, however, no further increase in the weight of the roots. Furthermore, chemical examination of the bark just above the girdle shows an extraordinary accumulation of carbohydrates. (This accumulation does not occur after girdling in the dormant season when no leaves are on the tree.) No accumulation of sugar occurs in the bark below the girdled region.

A more direct demonstration of the pathway taken by organic compounds in the plant is shown in Fig. 14.14. A leaf was supplied with radioactive water (^{3}HOH) and allowed to carry on photosynthesis for 30 min. At the end of this time, the radioactivity in the petiole (through which both xylem and phloem pass from leaf blade to stem) was localized in the sieve tubes and companion cells.

The transport of food materials through the phloem proceeds quite efficiently. Some fruits, such as the pumpkin, receive over half a gram of

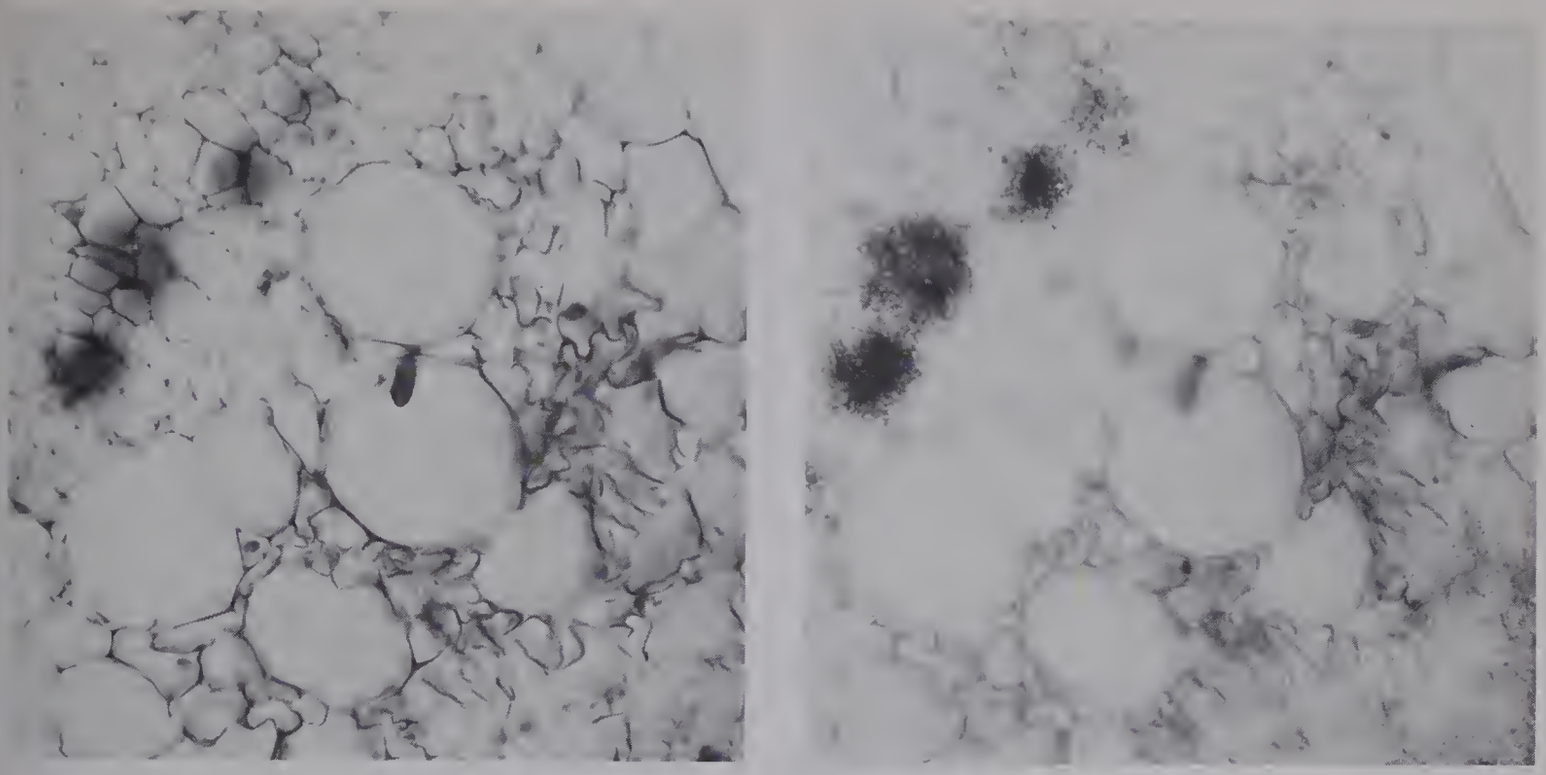


Fig. 14.14 A demonstration by radioautography that the products of photosynthesis are transported in the phloem. A cucumber leaf was supplied with radioactive water (^3HOH) and allowed to carry on photosynthesis for 30 min. Then slices were cut from the petiole of the leaf and covered with a photographic emulsion. Radioactive products of photosynthesis darkened the emulsion where it was in contact with the phloem (upper left), but not where it was in contact with the xylem vessels (center). In the photomicrograph on the left, the microscope is focused on the tissue in order to show the cells clearly; on the right, the microscope has been focused on the photographic emulsion. (Courtesy of R. S. Gage and S. Aronoff.)

food each day through the phloem. Other fruits have been found to do even better. Bearing in mind that the food is being transported in a fairly dilute water solution, an appreciable flow must occur. In fact, studies with radioactive tracers have revealed that substances can travel through as much as 100 cm of phloem in an hour.

Velocity of transport through the phloem

14.16 MECHANISM OF FOOD TRANSPORT

The mechanism by which sugars and other molecules manufactured by the plant are translocated through the phloem is not yet understood. It seems to be dependent upon the metabolic activity of the phloem cells, because any condition that slows down their metabolism also slows down the rate of translocation. Figure 14.15 illustrates apparatus with which one can show the effect of lowered temperature on the rate of food translocation. Oxygen lack similarly depresses it. Anything that kills the phloem cells puts an end to the process.

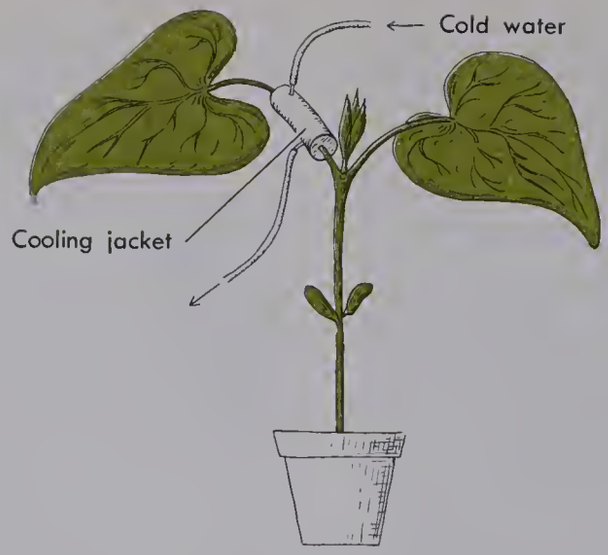


Fig. 14.15 Lowering the temperature of the petiole reduces the rate at which food is translocated out of the leaf.

The pressure-flow hypothesis

Several theories have been proposed to explain the movement of food materials through the phloem. According to one of these, water containing the food molecules in solution flows under **pressure** through the phloem. The pressure arises from the difference in water concentration of the solution in the phloem and the relatively pure water in nearby xylem ducts. With the accumulation of sugars and other products of photosynthesis in the phloem, water enters by osmosis (Fig. 14.16). Pressure thus builds up in these cells something like root pressure in reverse. As the sap is pushed down (and up) the phloem, sugars are removed by the cortex of both stem and root and consumed or converted into starch. Starch is insoluble and exerts no osmotic effect. Consequently, the osmotic pressure of the contents of the phloem decreases. Finally, relatively pure water is left in the phloem, and this is thought to leave by osmosis and/or be drawn back into nearby xylem vessels by the suction of transpiration pull.

Supporting evidence

How well does this theory fit the observed facts? According to it, the contents of the sieve tubes must be under pressure. This has been found to be true. When sieve tubes are punctured by such sap-eating insects as aphids, the sap flows through the insect's mouth parts and into its body without further assistance. In fact, if the body of the aphid is cut away from its mouth parts, sugar-rich sap continues to ooze from the cut end (Fig. 14.17). This theory also requires that the osmotic pressure in the phloem of the leaves be greater than in the phloem of food-receiving organs such as the roots. Generally this seems to be true although apparent exceptions have been found.

Evidence that weakens the case

On the other hand, one would predict on the basis of this theory that factors affecting the rate of metabolism of the *phloem cells* themselves would have no effect on translocation. The reason is that metabolic energy is needed only at the "ends" of the system, i.e. for depositing sugars in the phloem of the leaves and withdrawing them again from the phloem of the root and the growing shoot (Fig. 14.16). But, as we have seen, transport through the phloem *is* affected by localized inhibition of its metabolism (Fig. 14.15). Furthermore, there is a real question as to whether the mea-

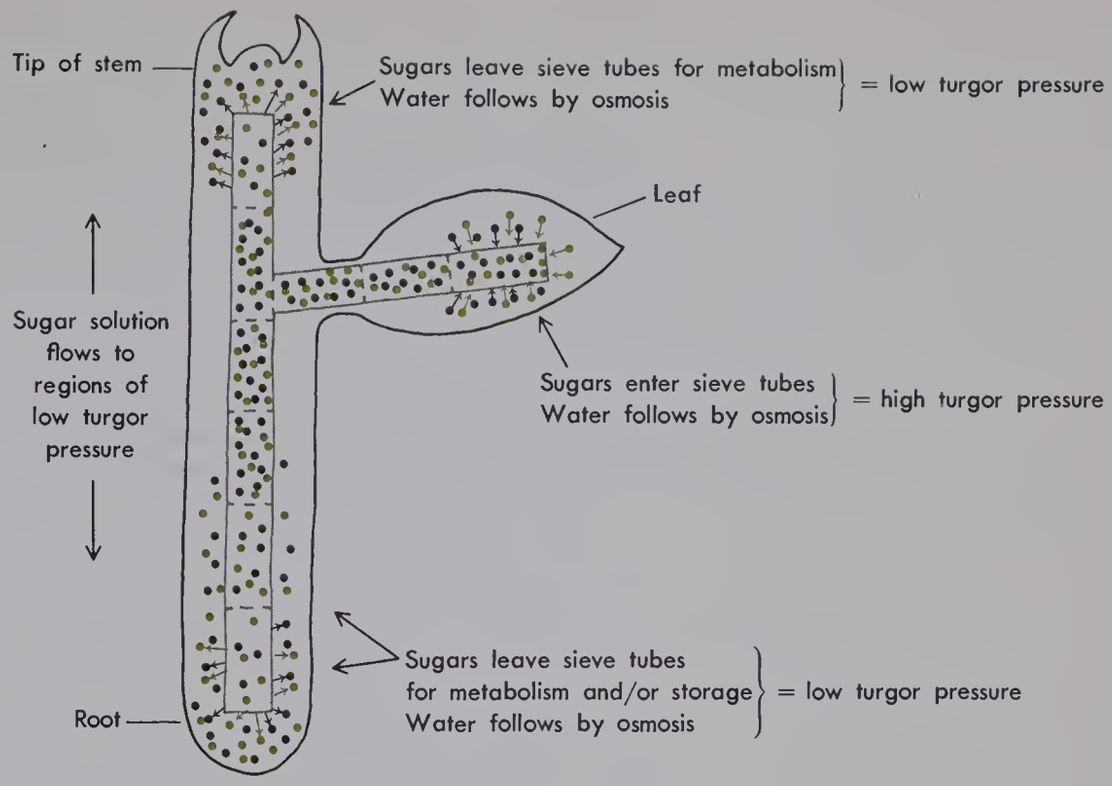


Fig. 14.16 The pressure-flow hypothesis of transport through the phloem. The pressure gradient between (1) the leaves and (2) the shoot and root drives the sap up and down through the sieve tubes. (Sugar molecules are shown in black; water molecules in color.)

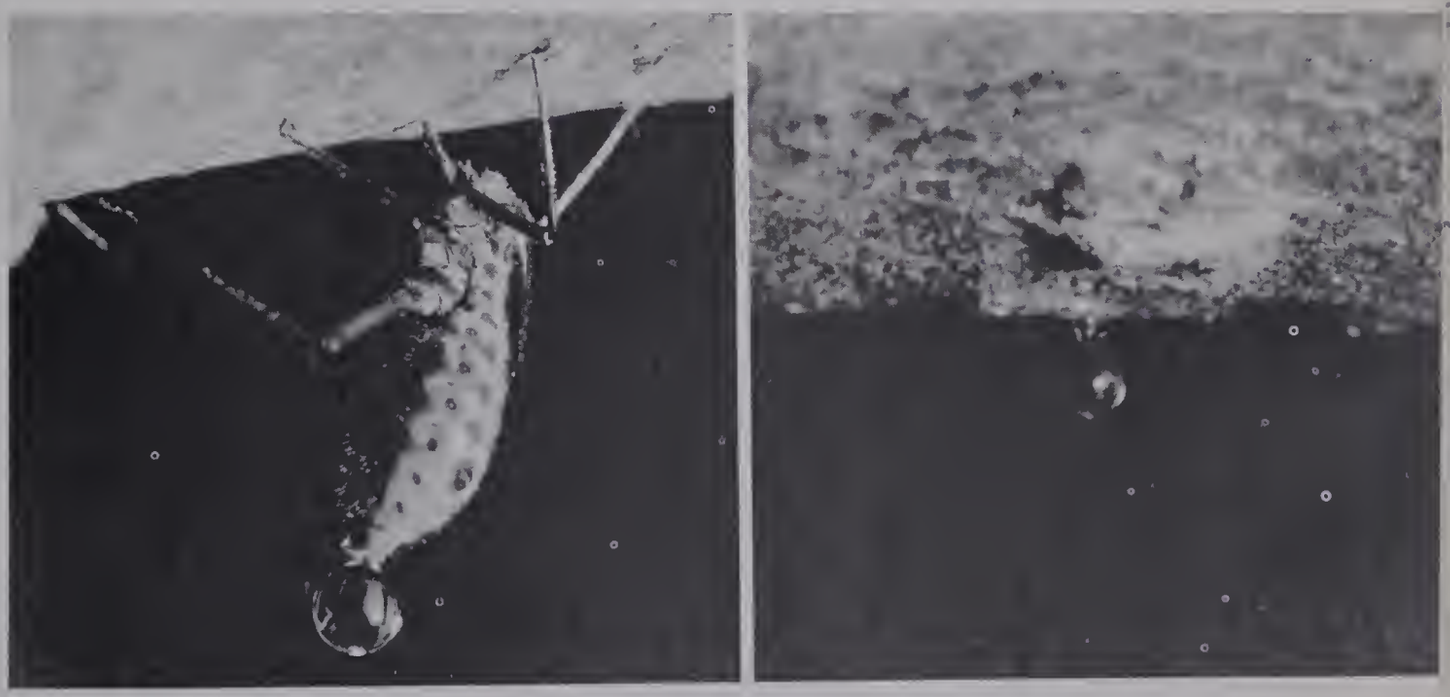


Fig. 14.17 Left: aphid feeding on the branch of a linden tree. Excess sugar is released as a drop of honeydew that serves as food for ants or bees. The sap in the phloem enters the insect's mouth parts under pressure. Right: it will continue to exude from the mouth parts after the aphid has been cut away from them. (Courtesy of Martin H. Zimmerman, *Science,* **133,** 73–79, Jan. 13, 1961.)

sured pressures are sufficient to push sap through the sieve tubes at the velocities that have been measured.

A number of alternative theories have been advanced which avoid the difficulties of the pressure-flow theory (but run into difficulties of their own). Several of these assume some sort of localized pumping mechanism working in relays along the length of the sieve tubes—perhaps at each sieve plate. Such theories *would* explain why chilling a restricted portion of the phloem inhibits translocation. As yet, however, no single theory has been proposed that adequately explains all the known facts of phloem transport. Such a theory still awaits fresh observations, better measuring techniques, new experiments and, especially, the creative imagination capable of welding all these into a comprehensive scheme.

Summary

Xylem and phloem represent one of the most important adaptations of plants to a land environment. In order to carry on photosynthesis, plants must expose a broad chlorophyll-containing surface to the sun's rays. For land plants, this means exposing a broad surface to the drying effect of the air. In order to remain functioning, the land plant must secure water for photosynthesis and replace water lost through transpiration with fresh water from the soil. As we have seen, the structure and functioning of the xylem permits this. On the other hand, the water-absorbing roots need food in order to remain alive. The phloem solves this transport problem. These two tissues have played a crucial role in the successful invasion by the vascular plants of almost all the exposed land on our planet.

EXERCISES AND PROBLEMS

1. Trace the path taken by a hydrogen atom from the time it enters a root hair incorporated in a molecule of water until it is stored in the root cortex incorporated in a molecule of starch.
2. In what ways does the circulatory system of a plant differ from that of a vertebrate?
3. Many low-growing plants secrete drops of water along the edges of their leaves. This phenomenon, called guttation, is particularly apt to occur during damp spring nights. Which of the forces by which water rises in the xylem do you think is responsible for guttation? Why?
4. What functions in the maple tree are carried out by roots?
5. Why is it good practice to prune off some of the branches of a tree or shrub that has just been transplanted?
6. If in counting the annual rings of an ancient elm, several narrow rings are found just within several wide ones, what conclusions might you draw?
7. What is cohesion? What role does it play in the upward transport of water?
8. What is adhesion? What role does it play in the upward transport of water?
9. What is the function of the upward transport of maple sap to the maple tree?
10. Summarize the factors that influence the rate of transpiration.

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15 EXCRETION AND HOMEOSTASIS

Excretion in Plants

Excretion in the Amoeba

Excretion in the Invertebrates

Structure of the Human Kidney

The Formation of Urine

Control of the Kidney

Mechanics of Elimination

Kidney Disease

Nitrogenous Wastes of Man

Excretion in the Other Vertebrates

In the preceding chapters of Part III, we have considered the ways in which living things (1) acquire materials (and energy) from their environment, (2) transport materials throughout their bodies, and (3) transform these materials within their cells. These operations are all part of an organism's metabolism. To complete our story of metabolism, we must now examine the ways in which living things return the waste products of their metabolism to the environment. In general, the most abundant products of metabolism are carbon dioxide, water, and ammonia. These substances should not, however, be considered simply as wastes. Each has important roles to play in the regulation of body activities (recall, for example, the effect of CO_2 on the rate of breathing) and in various anabolic cell syntheses. Even ammonia, a very toxic substance, is an essential reactant in a number of biochemical transformations of amino acid metabolism. It is, then, only as these substances accumulate to levels *in excess of the organism's needs* that they must be eliminated. The return of these unneeded products of metabolism to the environment is called **excretion.** As we have already studied (in Chapter 12) the ways in which plants and animals return gaseous wastes to the environment, we shall not stress these in the present discussion.

Excretion is the return of unneeded products of metabolism to the environment

15.1 EXCRETION IN PLANTS

There are several reasons why the excretory requirements of plants are less burdensome than those of animals

Excretion in plants does not pose any serious problems. There are several reasons for this. First, the rate of catabolism in plants is generally much lower than in animals of the same weight. Consequently, metabolic wastes accumulate more slowly. Second, green plants use much of the waste products of catabolism in their anabolic processes. Water and carbon dioxide, produced by respiration, are used in photosynthesis. Waste nitrogen compounds, whose excretion makes such demands upon animals, can be used by green plants in the synthesis of new protein. Finally, the metabolism of plants is based mainly on carbohydrates rather than proteins. This reduces their excretory needs, as the end products of carbohydrate metabolism are far less poisonous than the nitrogenous wastes produced by protein metabolism. Of course, plants do produce protein from which a variety of important cell structures and all their enzymes are made. Nevertheless, protein metabolism plays a much smaller role in plants than in animals, whose general body structure is based to such a great extent upon protein.

Among the aquatic plants, metabolic wastes are free to diffuse from the cytoplasm into the surrounding water. No cell is far removed from the water and the concentration of wastes within the cell exceeds the concentration of these substances in the water. The only product of metabolism for which this is not true is water itself. As we saw in Section 6.4, freshwater plants live surrounded by a water concentration greater than that within their cytoplasm. Not only does this eliminate diffusion (osmosis) as a means of getting rid of waste water, but (and far more important) it also exposes them to a continual inflow of water from the environment.

As water enters the cell by osmosis, the pressure within the cell rapidly increases. The rigid cellulose cell wall withstands this pressure and turgor results. When the pressure within the cell becomes equal to the osmotic pressure, equilibrium is achieved between the cell contents (especially the central vacuole) and the environment.

Among the terrestrial plants, the waste products of metabolism, such as salts and organic acids, are simply stored in the plant. These wastes may be stored in solid form in crystals or they may be dissolved in the fluid of the central vacuoles. In herbaceous species, the wastes simply remain in the cells until the tops of the plants die in the autumn. In perennial plants, wastes are deposited in the nonliving heartwood and are also eliminated when the leaves are shed.

15.2 EXCRETION IN THE AMOEBA

The amoeba and many other single-celled organisms live in a watery environment and eliminate their metabolic wastes by diffusion, just as the aquatic plants do. For many, the chief end product of protein metabolism is ammonia. This substance (in the form of its ion, NH_4^+) diffuses readily out of the cell before reaching a dangerous concentration.

However, these organisms cannot handle excess water in this way. Lacking a rigid cell wall, they cannot combat the continual inflow of water by building up turgor either. As we have seen (Section 6.4), the problem is solved by the **contractile vacuole.** Energy is used to force water back out of the cell and into the surrounding water. The contractile vacuole probably plays no significant role in the excretion of other substances. Amoebae that live in an isotonic environment (marine and parasitic forms) do not have a contractile vacuole. When placed in a hypotonic environment, however, they may develop one. This evidence suggests that the contractile vacuole serves simply to regulate water balance within the organism.

Contractile vacuoles maintain water balance

15.3 EXCRETION IN THE INVERTEBRATES

The excretory system of the earthworm is a particularly interesting one because in its structure and function it resembles the excretory apparatus of many other animals, both invertebrate and vertebrate. This does not necessarily mean that all these animals have inherited the structure from a common ancestor. It may be that such an efficient mechanism has arisen more than once in the course of animal evolution.

The structure and function of nephridia

In the earthworm and many other invertebrates, these excretory structures are called **nephridia** (Fig. 15.1). There is a pair of these in each segment of the earthworm (except the first three and the last). Each nephridium consists of a ciliated funnel, the nephrostome, which is mounted on the septum, the membrane that separates the segments. It leads through the septum into a long tubule which lies coiled in the next posterior segment. The far end (distal end) of the tubule enlarges to form a tempo-

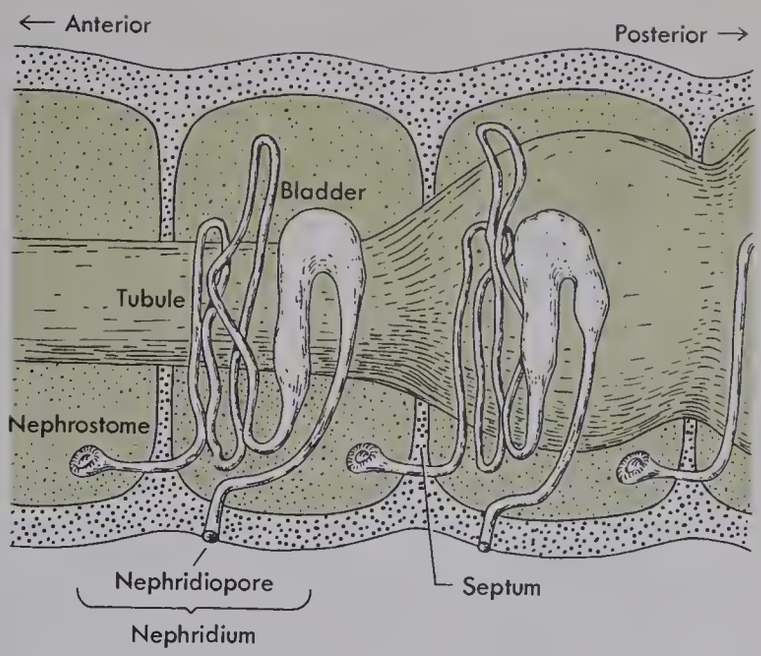


Fig. 15.1 Excretory system of the earthworm. A second nephridium is present in each segment. The nephridia pick up fluid containing both wastes and useful materials, but the useful materials are reclaimed as the fluid moves through the tubule.

rary storage structure, the bladder. The bladder opens to the outside through a pore, the nephridiopore, on the ventral surface of the worm.

The nephrostome lies within the body cavity. This cavity is filled with fluid which is chiefly lymph filtered from the closed circulatory system. This fluid is the ECF of the cells of the earthworm's body. It contains useful materials (e.g. glucose) and useless or harmful materials (e.g. nitrogenous wastes). The fluid enters the nephrostome and passes down the tubule. It is pushed along by cilia and muscular contractions of the tubule. As it moves along, materials useful to the worm are reclaimed by the cells lining the tubule. The tubule is richly supplied with blood vessels so that these useful materials are quickly placed back in circulation. Useless or waste materials are not reclaimed and they ultimately leave the worm by way of the nephridiopores. We shall see that many other animals solve their excretory problems in a similar way. *All* the components of the ECF are sorted over, the useful materials being reclaimed while the rest are not.

Tubular excretion

In addition to this indirect sort of excretory activity, the nephridia may also function in a more positive fashion. Secretory cells in the tubule probably can transfer wastes from the blood capillaries directly into the cavity of the tubule. This is called tubular excretion.

Among fresh-water animals, there are adequate quantities of water to flush away toxic nitrogenous wastes as fast as they accumulate. In fact, the chief problem of these animals seems to be coping with the problem of too much water entering their bodies from the hypotonic environment. Among terrestrial animals, the situation is quite different. Every effort must be made to conserve water. In the terrestrial arthropods, such as the insects, we get our first glimpse of the problems of excretion in a dry environment. The grasshopper solves the problem by means of both structural and biochemical modifications. The nitrogenous waste of the grasshopper (and other insects) is **uric acid.** This biochemical adaptation is

extremely important because uric acid combines high nitrogen content with low toxicity. This low toxicity is partly a result of low solubility. Just as soon as the concentration of uric acid begins to increase appreciably, it settles out of solution as a solid precipitate. As a solid, it exerts no biochemical effect and can be removed from the body with only a small amount of water.

Excretion in insects

Structurally, the insect's excretory system is made up of **Malpighian tubules.** These lie in the hemocoel and are bathed in blood (Fig. 15.2). Although there is no direct opening to the blood, the cells of the tubules extract soluble substances from it and pass them into the cavity of the tubules. Then valuable materials, including water, are reabsorbed. As the water concentration within the tubule decreases, uric acid precipitates. Instead of opening directly to the outside, the Malpighian tubules lead into the intestine where still more water is reabsorbed. The uric acid then passes to the outside with the feces. This excrement is quite dry. Thus the grasshopper is able to dispose safely of its nitrogenous wastes without losing valuable water in the process.

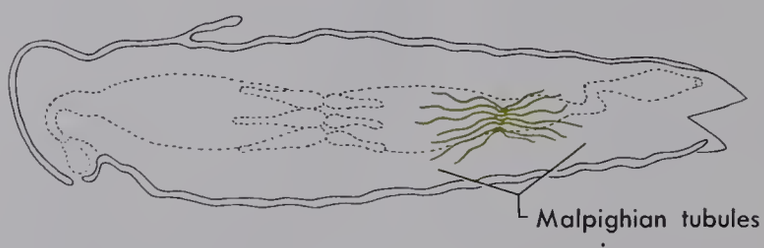


Fig. 15.2 The excretory wastes (chiefly uric acid) of the grasshopper are deposited in the intestine, where almost all moisture is reabsorbed. The grasshopper can thus excrete its metabolic wastes with practically no loss of water.

EXCRETION IN MAN

The main excretory organs of the vertebrates are the kidneys. Because more is known about the function of the mammalian kidney than any other type, we shall first examine it, using man as an example. Later in the chapter we shall examine some of the structural and functional modifications found in the kidneys of other vertebrates.

15.4 STRUCTURE OF THE HUMAN KIDNEY

The human kidneys are two bean-shaped organs each about the size of a clenched fist. They are located against the dorsal body wall on either side of the backbone.

Blood supply to the kidney

Although the total weight of the kidneys is only 0.5% of the weight of the body, the kidneys receive an extraordinarily rich supply of blood. Twenty to twenty-five percent of the blood pumped by the heart each minute flows through them. This blood reaches the kidneys by way of a right and left renal artery and leaves by the right and left renal veins (Fig. 15.3).

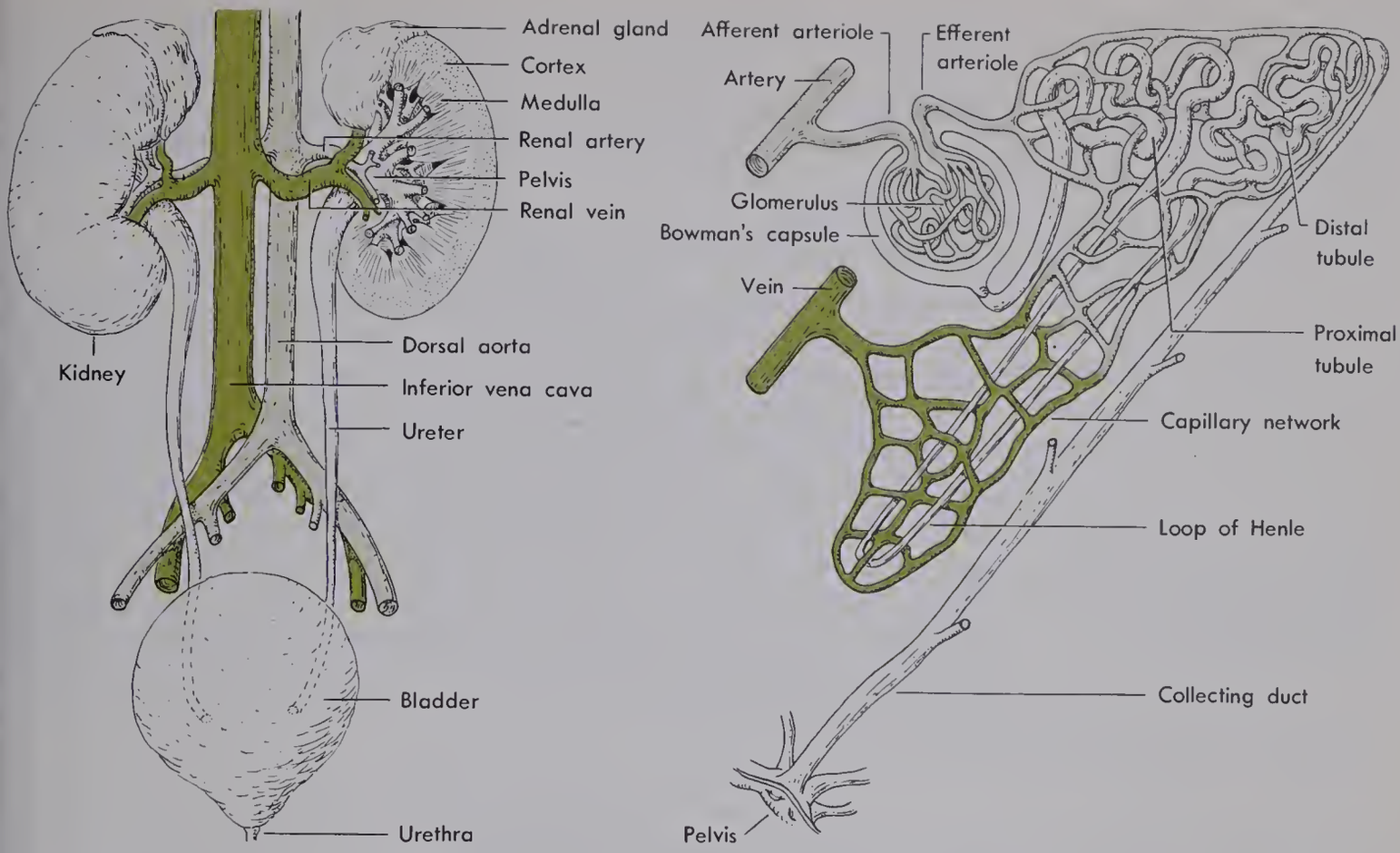


Fig. 15.3 Human excretory system. Left: gross anatomy. Right: a single nephron (enlarged). There are approximately one million nephrons in each kidney.

A transverse section of the kidney shows it to be composed of three distinct regions. The outer region is the **cortex.** Beneath this is the **medulla.** Within the medulla is a hollow chamber, the **pelvis** of the kidney.

The nephron is the structural and functional unit of the kidney

The cortex and medulla of each kidney are made up of approximately one million nephrons. The **nephron** is the structural and functional unit of the kidney. To understand the physiology of the kidney as a whole we need only learn the physiology of a single nephron. This is not as easy as it may sound. Only by inserting tiny needles into various parts of a single nephron and analyzing the fluid there has it been possible to deduce a fairly complete picture of the functioning of the nephron.

Structure of the nephron

The nephron consists of a long (about 40 mm) coiled tubule closed at one end and open at the other (Fig. 15.3). At the closed end of the tubule, in the cortex, the wall of the nephron is expanded and folded into a double-walled chamber, the **Bowman's capsule.** Within the infolded portion of the Bowman's capsule is a network of capillaries, the **glomerulus.** The tubule itself consists of three distinct segments. The first segment, the **proximal tubule,** is coiled near the Bowman's capsule. The cells of which its walls are constructed are richly supplied with mitochondria. Many microvilli extend from these cells into the interior (the lumen) of the

tubule. The proximal tubule leads to a long, thin-walled segment, the **loop of Henle.** This portion runs down into the medulla, makes a hairpin turn and returns to the region of the Bowman's capsule. Here the tubule expands again to form the **distal** (far) **tubule.** Like the proximal tubule, it is highly coiled.

15.5 THE FORMATION OF URINE

The nephron manufactures **urine.** It does this by filtering the blood and then reclaiming useful materials back into the blood. This leaves the useless material to pass out of the nephron in a solution which we call urine.

Each glomerulus receives blood from an afferent arteriole and discharges its blood into an efferent arteriole. The blood within the glomerulus, like that at the arteriole end of any capillary, is under pressure from the contraction of the left ventricle. This pressure causes water and the small molecules present in the blood (thus excluding proteins) to filter through the capillary walls. The fluid produced is called **nephric filtrate.** A glance at Fig. 15.4 will show that it is simply blood plasma minus almost all of the blood proteins. Essentially, then, it is no different from interstitial fluid (see Section 13.16).

Nephric filtrate is formed in the same way as interstitial fluid

Composition of plasma, nephric filtrate, and urine
gm/100 ml of fluid

Component	Plasma	Nephric filtrate	Urine	Concentration
Urea	0.03	0.03	2.0	60X
Uric acid	0.004	0.004	0.05	12X
Glucose	0.10	0.10	none	—
Amino acids	0.05	0.05	none	—
Total inorganic salts	0.72	0.72	1.50	2X
Proteins and other colloids	8.00	none	none	—

Fig. 15.4

Solutes are actively reclaimed in the proximal tubule

The nephric filtrate collects within the Bowman's capsule and then passes into the proximal tubule. Here the reabsorption of glucose, amino acids, vitamins, hormones, and a large quantity of inorganic ions (Na^+, K^+, Ca^{++}, Cl^-, HCO_3^-, $PO_4^{\equiv}$, $SO_4^{=}$) takes place. This reabsorption proceeds by active transport. All the details of how this is accomplished are not yet known, but enzymes and ATP are required. As mentioned above, the cells of the proximal tubule are richly supplied with mitochondria. Their rate of metabolism is equal to that of any cell in the body, including active muscle cells. Furthermore, their microvilli greatly increase the surface area exposed to the nephric filtrate. Thanks largely to the microvilli, the total surface area that participates in urine formation in man is over 60 square feet or three times the surface area of the exterior of the body.

The materials reabsorbed by the proximal tubule are returned to the blood system by a capillary bed which surrounds the tubules. In the mammals, this capillary bed receives its entire blood supply from the

efferent arteriole of the glomerulus. The bed is drained by venules which lead into the renal vein.

Water is passively reclaimed from the proximal tubule

As the various solutes are removed from the nephric filtrate and returned to the blood, a great deal of water follows them. This reabsorption of water is due entirely to the passive process of osmosis. The transfer of solutes from the nephric filtrate to the blood increases the water concentration of the nephric filtrate. Water then passes by osmosis into the blood and restores osmotic equilibrium.

Although the amount of nephric filtrate produced by a single nephron is not great, the quantity produced by two million of them is truly impressive. About 180 quarts of nephric filtrate are produced in the kidneys each day. This volume of fluid may represent more than twice the weight of the individual. About 80% of the water in the nephric filtrate is reabsorbed by the proximal tubules. Up to a pound of sodium bicarbonate, two and a half pounds of sodium chloride, a third of a pound of glucose and lesser amounts of other useful materials are absorbed along with the water. It is easy to see that life could not be sustained for very long if this amount of material simply passed out of the body.

Not all of the solutes in the nephric filtrate are reclaimed by the proximal tubule. Some 60% of the nitrogenous waste, urea, remains behind. Substantial quantities of salts also remain dissolved in the fluid within the tubule.

As this fluid passes into the loop of Henle, it is approximately isotonic with the blood. The solutes and water have been absorbed in equivalent proportions by the proximal tubule. This situation may be altered in the distal tubule. Here a more selective, precisely-regulated reabsorption of materials takes place. Additional quantities of salts and additional quantities of water may be absorbed. Furthermore, the exact amount of each substance reclaimed in the distal tubule is directly related to the level of that substance in the bloodstream. If the water content of the blood is lower than normal, additional quantities of water will be absorbed from the distal tubule until the concentration in the blood regains the normal value. So, too, with the various salt ions. The reabsorption of Na^+ ions in the distal tubule is particularly interesting, because H^+ ions can be exchanged for them. In other words, the distal tubule helps to preserve a constant pH in the blood by exchanging H^+ ions for Na^+ ions when the acidity of the blood tends to rise. In the rare instances when the blood becomes too alkaline, the distal tubule also acts to correct this. Consequently, in helping to maintain the pH of the blood within the normal limits of 7.3–7.4, the kidney can produce a urine whose pH drops as low as 4.5 or rises as high as 8.5.

Several adjustments to the composition of the ECF take place in the distal tubules

Tubular excretion supplements the filtration-absorption mechanism

Although urine formation in man occurs primarily by the filtration-reabsorption mechanism just described, an auxiliary mechanism is also involved. This is **tubular excretion.** The cells of the tubules remove certain substances from the blood in the surrounding capillaries. These substances are then excreted directly into the interior of the tubules and added to the urine. In all probability this process is of little importance in man. A

nitrogenous waste, creatinine, and potassium ions are two of the few normal plasma constituents to be excreted in this fashion. Even without tubular excretion, the kidney could probably handle the elimination of these substances easily. Physiologists have discovered that tubular excretion does play an important role in the elimination of certain abnormal constituents, such as penicillin and some other drugs.

15.6 CONTROL OF THE KIDNEY

We do not know yet just what controls the selective reabsorption of most materials in the distal tubule. In some cases our chemical regulators, the hormones, are involved. A hormone that regulates the reabsorption of Ca^{++} in the distal tubule and one that regulates the reabsorption of Na^{+} have been discovered. The details of their activity will be examined in Chapter 25.

Vasopressin regulates water reabsorption in the distal tubules and collecting ducts

Still another hormone, **vasopressin,** has been found to regulate the reabsorption of water in the tubules and also in the collecting ducts that drain them (Fig. 15.3). Vasopressin is secreted by a gland located at the base of the brain, the pituitary gland. The secretion of this hormone is controlled by the water concentration of the blood. Anything which tends to dehydrate the body, such as perspiring heavily, causes a drop in the water content of the blood. This is detected by special receptors in the brain which stimulates the pituitary gland to release vasopressin. Vasopressin is then carried by the blood to the kidney. There it increases the reabsorption of water. This tends to restore the normal water level in the blood. It also leads to the production of a scanty, concentrated urine. When vasopressin is being actively secreted, the concentration of solutes in the urine may become as much as four times that of the blood. Man shares this ability to produce a hypertonic urine with the other mammals and the birds. No other vertebrates can do it.

Only mammals and birds can produce a hypertonic urine

On the other hand, if the blood starts to become too dilute (as would occur after drinking a large quantity of water), the secretion of vasopressin is inhibited. The tubules and collecting ducts fail to absorb as much water, and a great deal of watery urine is produced. The concentration of solutes in this urine may be as little as one-fourth that in the blood.

Occasionally people lose the ability to secrete vasopressin. They become victims of the disease **diabetes insipidus.** As you might guess, the most dramatic symptom of this ailment is an enormous production of watery urine. It is accompanied by terrible thirst. Sufferers may urinate as much as 20 liters of urine each day (10 to 20 times the average amount). To avoid fatal dehydration, they must constantly replace this water loss by drinking fresh water.

The kidney is one of the major homeostatic devices of the body

Our study of the physiology of the tubules should make it quite clear that the kidney is far more than just an excretory organ. Certainly it removes nitrogenous and other wastes. However, it also removes foreign substances from the blood and normal plasma constituents that are present in the blood in greater than normal concentration. When excess water,

sodium ions, chloride ions, etc. are present, the excess quickly passes out in the urine. On the other hand, the kidney steps up its reclamation of these same substances when they are present in the blood in less than normal concentration. In this way, the kidneys continually regulate the chemical composition of our blood within very narrow limits. Excretory products are eliminated simply because they naturally tend to accumulate to excess levels. The maintenance of a constant blood composition in turn assures a stable lymph. In short, the kidney is one of the most important of our organs for maintaining constant the composition of the internal environment. It is one of the major homeostatic devices of the body.

15.7 MECHANICS OF ELIMINATION

Pathway of urine

The process of urine formation continues unceasingly. When the urine leaves the distal tubule, it flows into a collecting duct, which passes through the medulla. The collecting duct drains urine from several nephrons into the pelvis of the kidney (Fig. 15.3). The urine then flows down from the kidney to the bladder by means of a duct, the **ureter.** The **bladder** is a hollow, muscular organ which expands as urine flows into it from the two kidneys. When the bladder becomes filled with urine, the muscular sphincter guarding its outlet relaxes and urine flows to the outside through the **urethra.** In the infant, relaxation of this sphincter occurs automatically. In older people, the sphincter is usually under conscious control.

The two ureters enter the bladder in such a way that their exits become forced shut when the pressure in the bladder begins to build up (Fig. 15.5). If urination is delayed for some time, urine begins to accumulate in the ureters and even in the pelvises of the kidneys. When the bladder is finally emptied, this accumulation is permitted to flow down. Thus the individual finds that a long-delayed urination is quickly followed by the need for a second.

15.8 KIDNEY DISEASE

As one would expect from the sheer magnitude and crucial importance of the job done by our kidneys, any prolonged interference with their func-

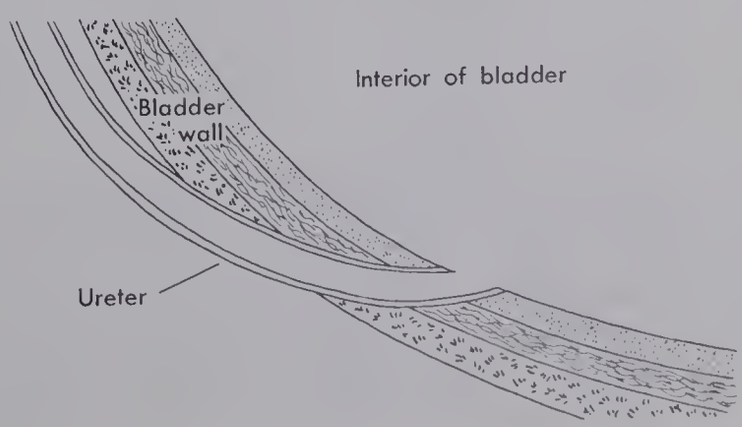


Fig. 15.5 Pressure within the bladder closes the ends of the ureters, causing urine to accumulate in them.



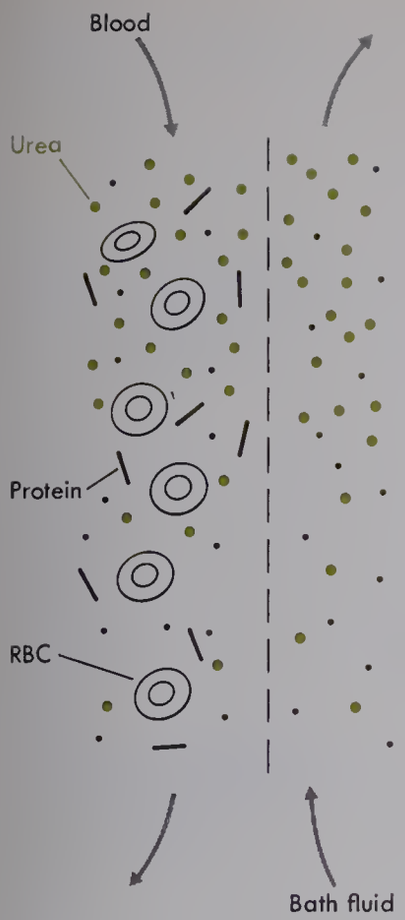
Fig. 15.6 An artificial kidney. Blood is withdrawn from an artery (upper tube), passes through a bubble trap, and enters the artificial kidney at the top. A continuous supply of fresh bath fluid (clear) enters the machine (lower left), passes over the dialysis membranes, and leaves (upper right) to be discarded. Purified blood leaves the artificial kidney (bottom), passes through a second bubble trap, and is returned to an arm vein (lower tube). Photo courtesy of the Cordis Corporation.

tioning has grave consequences. Unfortunately, there are a number of diseases which result in reduced or even no kidney function. Shock from serious injury and/or excessive blood loss, certain poisons, and the aftermath of certain infectious diseases may bring on a period of acute kidney failure. If the victim can survive the period of shutdown—with its attendant buildup of toxic wastes—the kidneys may begin functioning again as well as before. On the other hand, there are a number of progressive, degenerative diseases of the kidney which lead to a gradual, irreversible loss of kidney function.

Kidney failure

Artificial kidneys

One weapon against these life-threatening diseases has been the development of artificial kidneys. These operate on the principle of dialysis (Fig. 3.12). As we saw in Section 6.3, a semipermeable membrane, e.g. a sheet of cellophane, separating two solutions permits small molecules and ions to pass through but blocks the passage of large molecules such as proteins. A kidney machine (Fig. 15.6) simply passes blood (treated with heparin, an anticlotting agent) across one side of a cellophane membrane



and a carefully prepared bath fluid across the other. The fluid cannot, of course, simply be water, because this would lead to a rapid loss of all small molecules and ions from the blood. However, by preparing a bath fluid of precise composition, any particular ion or molecule can be removed from (or added to) the blood, as appropriate. In fact, the efficiency of exchange is so great that if no urea is present in the initial bath fluid, the machine will remove urea from the blood more rapidly than normal kidneys can.

Artificial kidneys have proved enormously effective in helping victims of acute kidney malfunction to survive the crisis until their own kidneys resume operation. They have also enabled many sufferers from chronic conditions to remain alive, though at an enormous expense of time (often three sessions of six or more hours per week), money, and psychological well-being.

A promising alternative to long-term dialysis for chronic kidney failure is the transplantation of a new kidney. The operation is technically quite simple. The donor kidney is placed low in the abdominal cavity and its artery and vein are connected to an iliac artery and vein respectively. The ureter can easily be connected to the bladder (Fig. 15.7).

The major problem with kidney transplants is the problem of immune rejection. Unless the patient has an identical twin as the donor, his immune system (to be examined in Chapter 30) will recognize the implanted kidney as "foreign" and proceed to destroy it. However, a variety of drugs have been found to be effective in suppressing the body's immune mechanism, and with the careful use of these many implanted kidneys remain functional for periods of several years. As for transplants between identical twins, there is no need for immunosuppressive drugs, and the new kidney should survive indefinitely (16 years is the record at the time of this writing) unless the same underlying disease process that destroyed the host's own kidneys proceeds to destroy the implant.

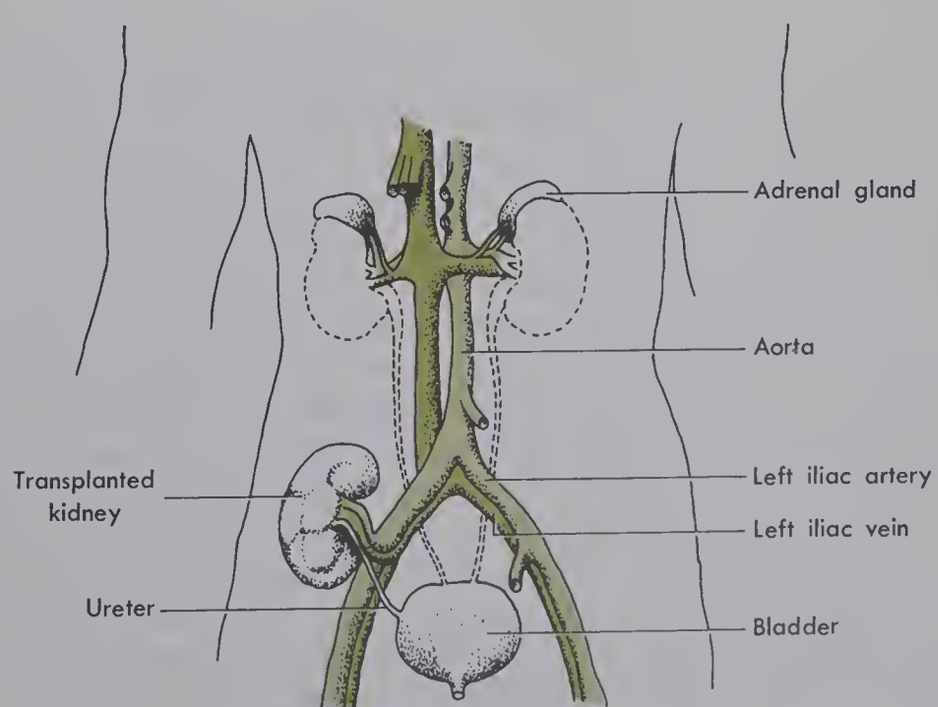


Fig. 15.7 Kidney transplant procedure. The recipient's own, diseased, kidneys are removed. The renal arteries and veins are tied off (except for the branches supplying the adrenal glands, which are left intact).

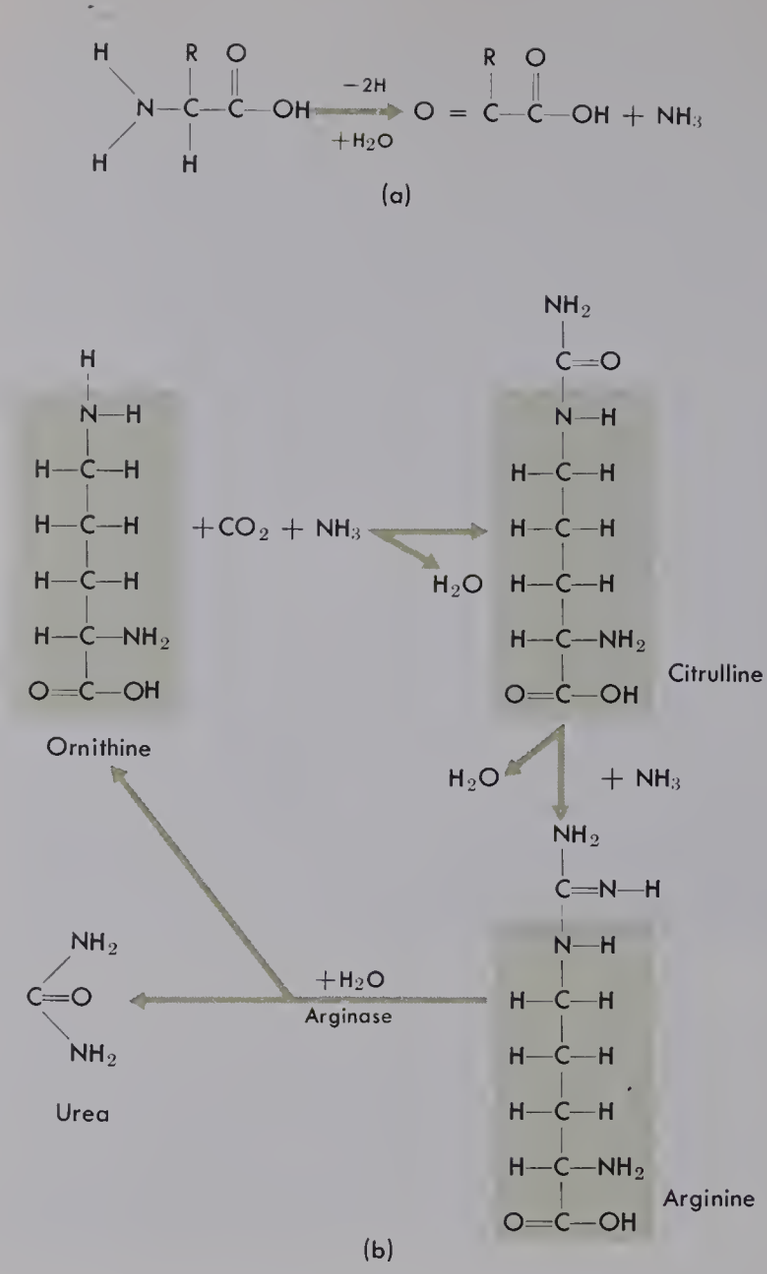


Fig. 15.8 (a) Deamination and (b) the synthesis of urea. The action of arginase on arginine produces a molecule of urea and regenerates ornithine for recycling.

15.9 NITROGENOUS WASTES OF MAN

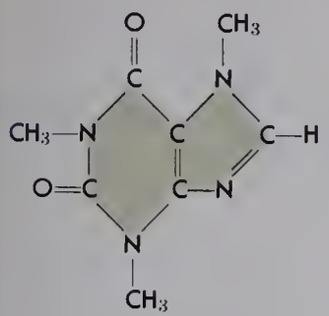
The urea cycle

The chief nitrogenous waste in man is urea. It is manufactured in the liver. Most of the nitrogenous wastes in the body arise from the breakdown of amino acids. This breakdown, **deamination,** also occurs in the liver. Deamination results in the accumulation of ammonia (Fig. 15.8). Ammonia is an extremely poisonous substance and its accumulation in the body would quickly prove fatal. Fortunately, the liver contains a system of carrier molecules and enzymes which quickly convert ammonia (and carbon dioxide) into urea. As each molecule of urea is synthesized, the carrier molecule, ornithine, is regenerated for re-use. Although our bodies cannot tolerate high concentrations of urea, it is much less poisonous than ammonia. As we have seen, the kidney removes it efficiently from the body.

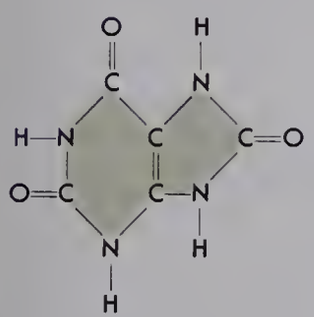
In mammals, uric acid is produced by the catabolism of nucleic acids

The presence of small quantities of uric acid in the urine is interesting. Uric acid is the main nitrogenous waste of insects, lizards and snakes, and birds. Of all the mammals, however, only man, the higher apes, and the

Fig. 15.9 Structural formulas of (a) caffeine and (b) uric acid.



(a)



(b)

Dalmatian dog excrete it. Our uric acid arises from the breakdown of nucleic acids rather than proteins. Other mammals produce uric acid by the breakdown of nucleic acids, but they also have an enzyme which then decomposes the uric acid. As mentioned earlier, uric acid is very insoluble. In some humans, the concentration of uric acid may be sufficiently high that it begins to precipitate out of solution. Needlelike crystals of uric acid accumulate in the joints, producing excruciating pain. The ailment is known as gout.

The uric acid molecule is quite similar in structure to the caffeine molecule (Fig. 15.9). Caffeine is the ingredient in coffee which, for many people, seems to be a mental stimulant. One scientist has suggested that when the loss of the ability to break down uric acid occurred in our early ancestors, their mental activities were heightened by the accumulation of this substance in the blood. Unquestionably, the dominant feature in the evolution of man has been the development of the brain. Did uric acid play a role in this? We do not know. However, the fact that many of the famous (and brilliant) men of history were known to suffer from gout is used as a support for this interesting speculation. Do you see any weaknesses in this notion?

EXCRETION IN THE OTHER VERTEBRATES

All vertebrates have kidneys. Like the human kidney, they are made up of masses of nephrons. There are, however, some differences in the structure and functioning of the nephrons in the various vertebrate kidneys. These differences can be related to the environment in which the animals live.

15.10 AQUATIC VERTEBRATES

The kidney of freshwater fishes maintains water balance

The freshwater fish, like all freshwater animals, suffers a continual inflow of water from its hypotonic environment. The scales of the fish are impervious to water, but the delicate gill membranes provide ready access for water to enter the body. In order to avoid serious dilution of its body fluids, the freshwater fish must excrete this excess water. It does this by substantially the same mechanism as the amoeba: the use of energy to force the water back into the environment. In the fish, ATP provides the energy for the contraction of the heart. The contraction of the heart provides the pressure to force blood out of the glomerulus into the Bowman's capsule. Of course, valuable solutes (e.g. salts, glucose) pass into the nephric filtrate, too. These must then be reclaimed by the tubules. They pass into the blood in the capillaries surrounding the tubules. The blood supply in these capillaries comes from the glomerulus (as in man) and also from the veins which drain the posterior portion of the fish's body (Fig. 15.10). This part of the blood supply is called the renal portal system. What other example have

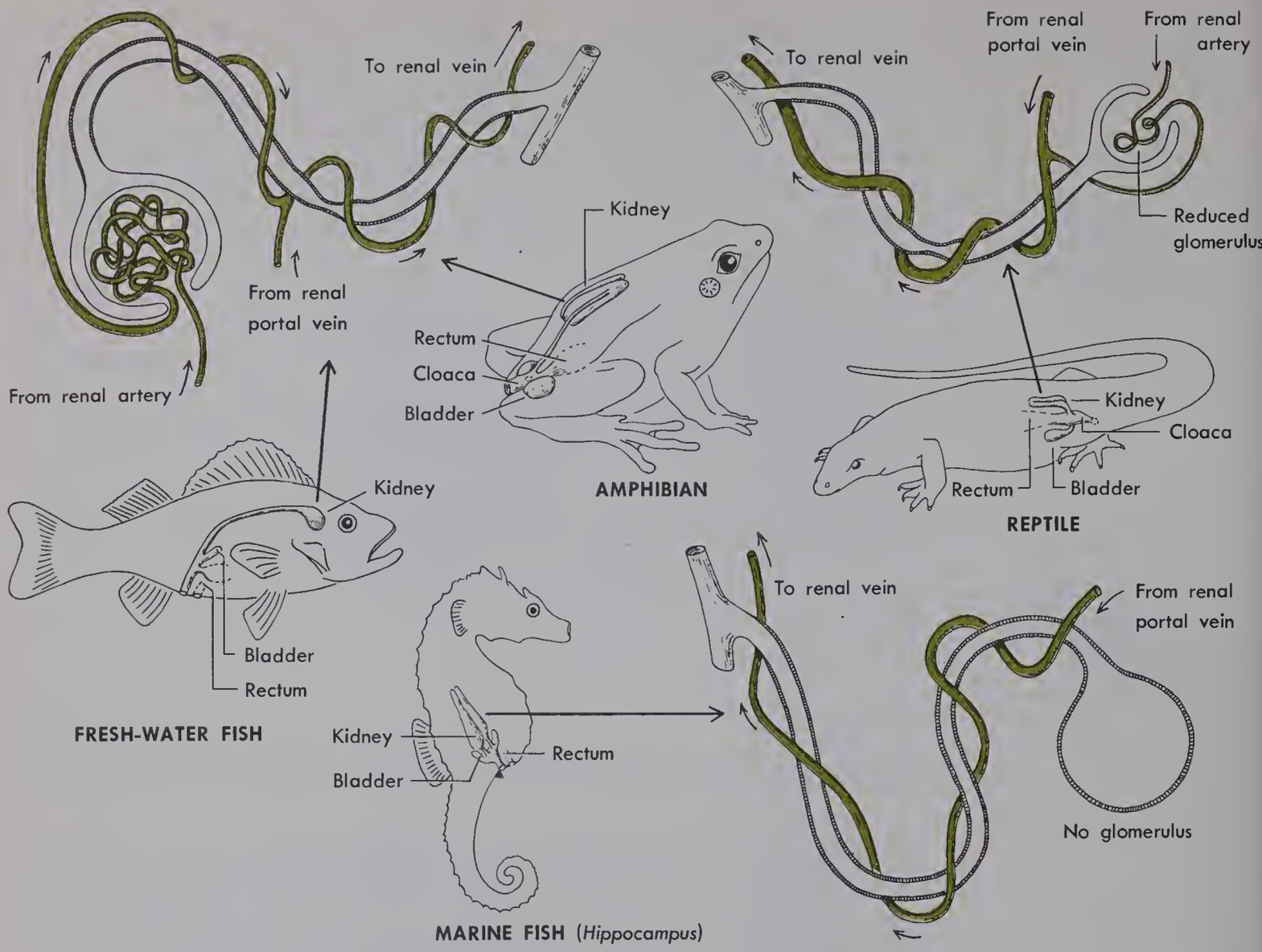


Fig. 15.10 A comparison of vertebrate kidneys. In each case, the structure of the nephron is related to the role it plays in maintaining a suitable water content within the animal.

you studied of a vein leading to a capillary bed rather than directly back to the heart?

After salt reabsorption is complete, the urine is little more than just water. Most of the nitrogenous wastes (including large amounts of NH_3) of the fish actually leave the body by diffusion out of the gills. The kidney is really a device for maintaining proper water balance in the animal rather than an organ of excretion.

The marine environment

Marine fishes face just the opposite problem from that of the freshwater fishes. The salt content of sea water is so high that, physiologically, the marine fishes actually live in a dry environment. They are in continual danger of loss of vital body water to the hypertonic environment.

The marine fishes have solved this problem in two ways. The so-called cartilaginous fishes, the sharks, skates, and rays, have developed a toler-

The cartilaginous fishes

ance to extraordinarily high levels of urea in their bloodstream. Shark's blood may contain 2.5% urea in contrast to 0.01–0.03% in other vertebrates. This urea level is so high that the shark's blood is isotonic with the sea water. It lives in osmotic balance with its environment and has a kidney which functions similarly to man's (with the exception that far more urea is reabsorbed in the shark's tubules than in man's).

Marine bony fishes

The marine bony fishes have solved the problem differently (Section 6.4). They lose water continuously but replace it by drinking sea water and desalting it. The salt is returned to the sea by active transport at the gills. Living in constant danger of dehydration by the hypertonic sea, the bony fish has no reason to pump out large quantities of nephric filtrate at the glomerulus. The less water placed in the tubules, the less water that has to be reabsorbed. It is not surprising that many of these fishes have very small, weakly functioning glomeruli. Some have no glomeruli at all (Fig. 15.10).

With a reduction in the filtration-reabsorption mechanism, these fishes rely more on tubular excretion for eliminating undesired solutes. Tubular excretion requires a good blood supply to the tubules. Lacking efficient glomeruli, the renal portal system must carry most of the burden.

15.11 TERRESTRIAL VERTEBRATES

The amphibian kidney can cope with either hypotonic or dehydrating conditions

The **amphibian** kidney, like that of the freshwater fish, functions chiefly as a mechanism for excreting excess water. The permeable skin of the frog provides an easy site for the surrounding water to enter by osmosis. As the name amphibian suggests, however, these animals may spend some time on land. The problem then becomes one of conserving water, not eliminating it. The frog adjusts to the varying water content of its environment by adjusting the rate of filtration at the glomerulus. A renal portal system is present to carry away materials reabsorbed by the tubules during those periods when the blood flow through the glomerulus is restricted (Fig. 15.10). Furthermore, the frog is able to use its urinary bladder as a water conservation device. When in the water, the frog's bladder quickly becomes filled with a copious, watery urine. On land this water is reabsorbed into the blood thus replacing water lost by evaporation through the skin. The reabsorption mechanism is under the control of a hormone quite similar to vasopressin.

Excretion in reptiles

Many **reptiles,** e.g. desert rattlesnakes, live in extremely dry environments. Among the many adaptations that enable them to do so is their ability to convert waste nitrogen compounds into uric acid. As we have noted, uric acid is quite insoluble. Therefore, it can be excreted without the use of too much water. Accordingly, the reptile glomeruli are quite small. In fact, some reptiles have no glomeruli at all. Those that do have glomeruli filter just enough fluid to wash the uric acid, which is excreted by the tubules, into the **cloaca.** (The cloaca is a chamber through which the feces and the sex cells, as well as urine, pass on the way to the outside. The name comes from the Latin word for sewer.) Within the cloaca, most of the water

is reabsorbed. Periodically, the reptile empties its cloaca. The feces are brownish in color; the uric acid forms a white paste. These water conservation mechanisms enable the reptile to survive even though little or no fresh water is available. The water content of its food coupled with the water produced in cellular respiration is usually sufficient for its needs.

It is important to note that the ability of these reptiles to convert waste nitrogen into uric acid is just as much an adaptation to dry-land living as their waterproof skin and their lungs. One is a physiological adaptation, the others are morphological. But note, too, that the excretion of uric acid is accompanied by structural changes in the kidney. Structure and function in living organisms are completely interrelated. One cannot appreciate the structural organization of cells, tissues, organs, and systems without understanding how they function. On the other hand, the physiological activities of living organisms can only be accomplished by properly organized structures. The student who studies the way in which living things are put together without any concern for the way these structural components function glimpses only half the story of life. Just as restricted is the view of the student who studies physiological activities with no concern for the structures which accomplish these.

Excretion in birds

Birds solve the problem of dry-land living in a way similar to that of the lizards and snakes. Uric acid is their nitrogenous waste product, too. It is the whitish material that pigeons leave on statues. Although their glomeruli are very small, they do function actively and a substantial amount of nephric filtrate is produced. There are probably two reasons for this. Birds (like mammals) have no renal portal system, so the blood needed for tubular reabsorption and excretion must come from the glomeruli. Furthermore, birds (again like mammals) have high blood pressures and thus cannot avoid substantial filtration rates at the glomeruli. However, the increased production of nephric filtrate is more than compensated for by an increased absorption of water in the tubules. The concentration of uric acid in bird urine may be as high as 21%. This is over 3000 times the concentration of uric acid in the blood. Tubular excretion of uric acid is important in this process of concentration, but the reabsorption of water in the tubules is vital, too.

The excretion of urea entails unavoidable losses of water

Although **mammals** are also terrestrial animals, their chief nitrogenous waste is urea. This substance requires a good deal more water for its elimination than does uric acid. As we have seen, mammals excrete large quantities of nephric filtrate but are able to reabsorb most of this in the tubules. But even with maximum reabsorption of water in the tubules, man must excrete several hundred milliliters each day in order to flush urea out of his body.

Some mammals have developed kidneys more efficient than man's in this respect. Water reabsorption in the tubules of the kangaroo rat (Fig. 15.11) is so effective that it can produce urine 17 times as concentrated as its blood. The most hypertonic urine that man can produce is only about four times as concentrated as his blood. The kangaroo rat also conserves water by remaining in its burrow during the heat of the day, thus reducing



Fig. 15.11 The kangaroo rat (*Dipodomys spectabilis*). This little mammal is so well adapted to life in the desert that it never needs to drink water. (Courtesy of Dr. Knut Schmidt-Nielsen.)

water loss through evaporation. This combination of behavioral and physiological adaptations makes it unnecessary for the kangaroo rat to drink water. The small amount of moisture in its food and the water produced by cellular respiration are fully adequate to its needs.

We have been discussing the various kidneys found in vertebrates of today. It is thought that all of these vertebrates have evolved over a vast period of time from a common ancestor. Probably the fishes have evolved the least in this period. The mammals and birds represent a much greater change from the earliest vertebrates. As the various vertebrate classes evolved on earth, new ways of life developed, too. The amphibians took to the land. The birds took to the air. It would be surprising if the evolution of an organ as important as the kidney did not reflect these changes. In this chapter we have seen how the various vertebrate kidneys are adapted to the way of life of their owners. In Chapter 37, we shall reexamine these adaptations as part of the broader picture of the many structural and functional changes that occurred during the evolution of the various classes of vertebrates.

EXERCISES AND PROBLEMS

1. What is the chief nitrogenous waste of the (a) rattlesnake, (b) dog, (c) chicken, (d) shark?
2. Name the chief nitrogen-excreting organs of the (a) earthworm, (b) grasshopper, (c) frog.
3. How does the kidney maintain homeostasis of the blood with respect to (a) Cl^- concentration, (b) urea concentration, (c) pH?

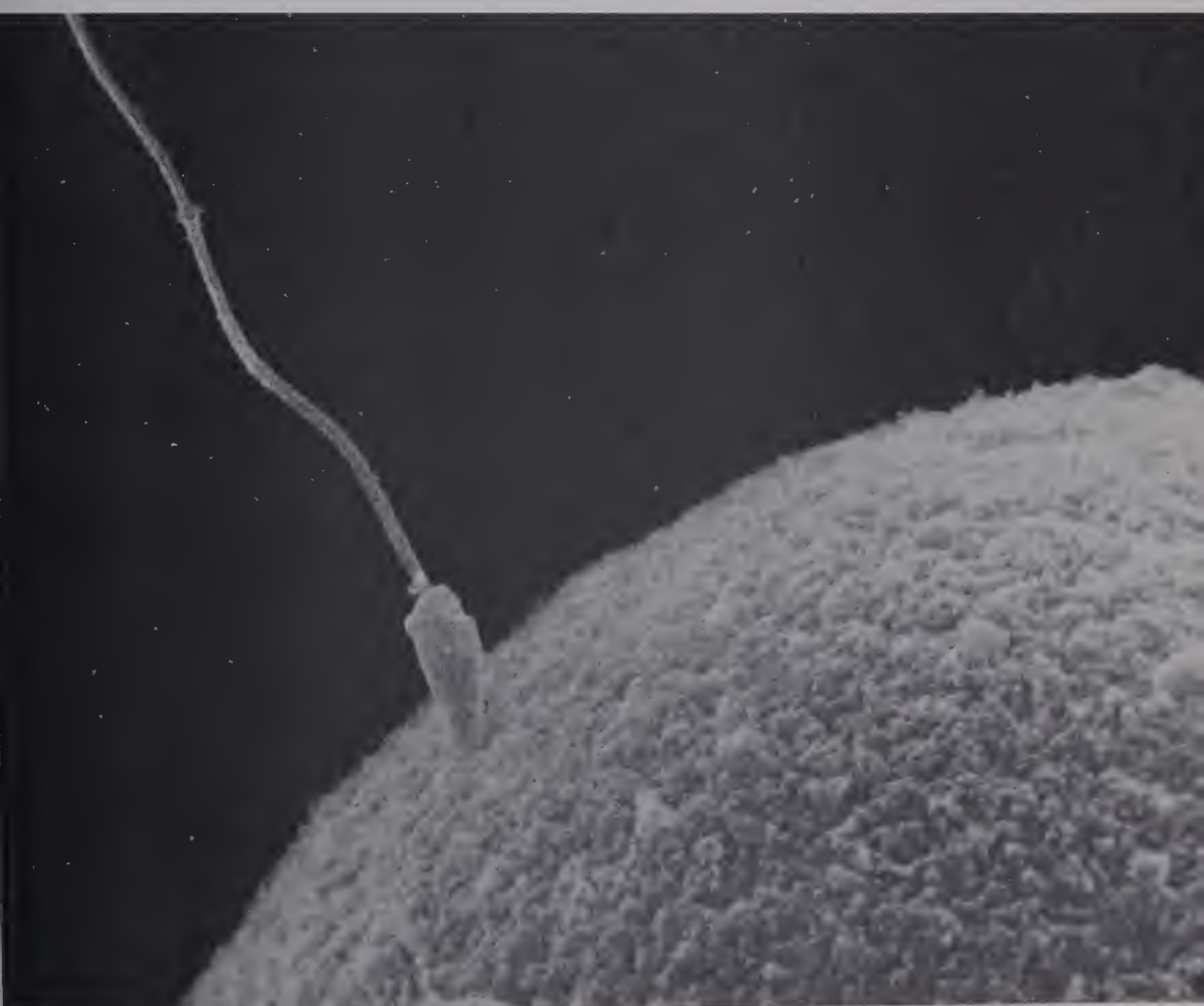
4. Trace the path traversed by a molecule of urea from the time it enters a renal artery until it leaves the body.
5. Do you suppose evergreens are able to eliminate wastes by shedding their leaves ("needles")?
6. Compare interstitial fluid and nephric filtrate with respect to (a) composition, (b) method of formation.
7. What substances are found in blood plasma but not in nephric filtrate?
8. Distinguish between ureter and urethra.
9. Synthesis of urea occurs in what organ?
10. Why is the problem of excretion less complex in the maple tree than in the dog?
11. List the most important structures for maintaining water balance in the (a) amoeba, (b) trout, (c) planarian, (d) man, (e) *Anacharis.*
12. How does the quantity of urine formed by a frog change when it moves from a pond onto land? Would the quantity of urine formed by a beaver change under the same circumstances?
13. Distinguish between excretion and egestion.
14. Carnivorous mammals excrete a higher concentration of urea than herbivorous ones. Explain.
15. During the formation of urine, why does the concentration of salts not increase in proportion to the concentration of urea?
16. Name four organs of man that have an excretory function.

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The moment of fertilization of a sea urchin egg as seen with the scanning electron microscope. (Courtesy of Dr. Don W. Fawcett.)

Part IV REPRODUCTION



16

THE REPRODUCTION OF CELLS AND ORGANISMS

Asexual Reproduction in Unicellular Organisms

Asexual Reproduction in Multicellular Organisms

The Nature of Asexual Reproduction

Mitosis

The Nature of Sexual Reproduction

Sexual Reproduction in Bacteria

Meiosis

One of the most important aspects of living things is their ability to reproduce their kind. For every organism, there comes a time when its powers of metabolism, growth, and responsiveness are insufficient to maintain its complex organization against other forces. Attack by predators, parasites, starvation, other harmful changes in the environment, or simply those ill-defined processes that we call aging, result ultimately in the death of the organism. However, the species survives for periods far greater than the lifetime of any individual in it. This is accomplished with the production of new individuals by the old before the old die. Many of the major questions in biology concern this ability of living things to produce copies of themselves.

Sexual vs. asexual reproduction

Two quite distinct methods of producing offspring are found among living things. One method is sexual reproduction. This is the production of new individuals that combine the hereditary information contributed by two different cells, generally representing two different parents. In most organisms, these cells are the gametes. The other method of reproduction involves only one parent. It is called asexual reproduction.

ASEXUAL REPRODUCTION

Asexual reproduction is the formation of offspring without the union of two gametes. Widely used by microorganisms, plants, and lower animals, it may be accomplished by a variety of specific methods.

16.1 ASEXUAL REPRODUCTION IN UNICELLULAR ORGANISMS

Fission

The most common method of asexual reproduction among unicellular organisms is **fission.** The organism divides into two roughly equal halves. Each of these then grows to full size and the process may then be repeated. Under ideal conditions, bacteria can divide by fission every 20 to 30 minutes. *Amoeba* and most of the other protozoans also reproduce by this method (Fig. 16.1).

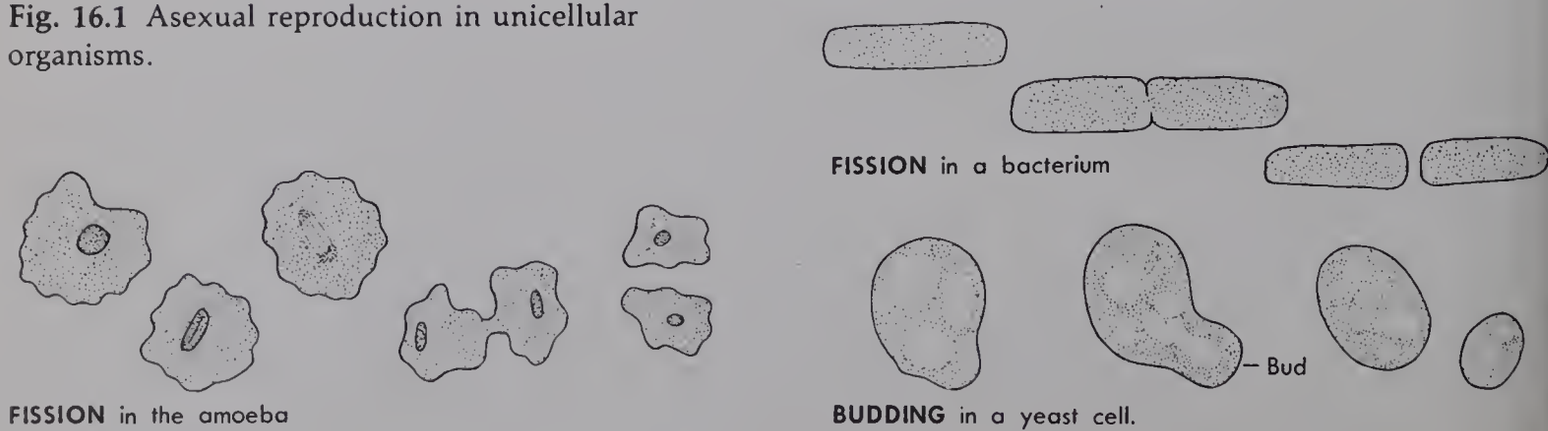


Fig. 16.1 Asexual reproduction in unicellular organisms.

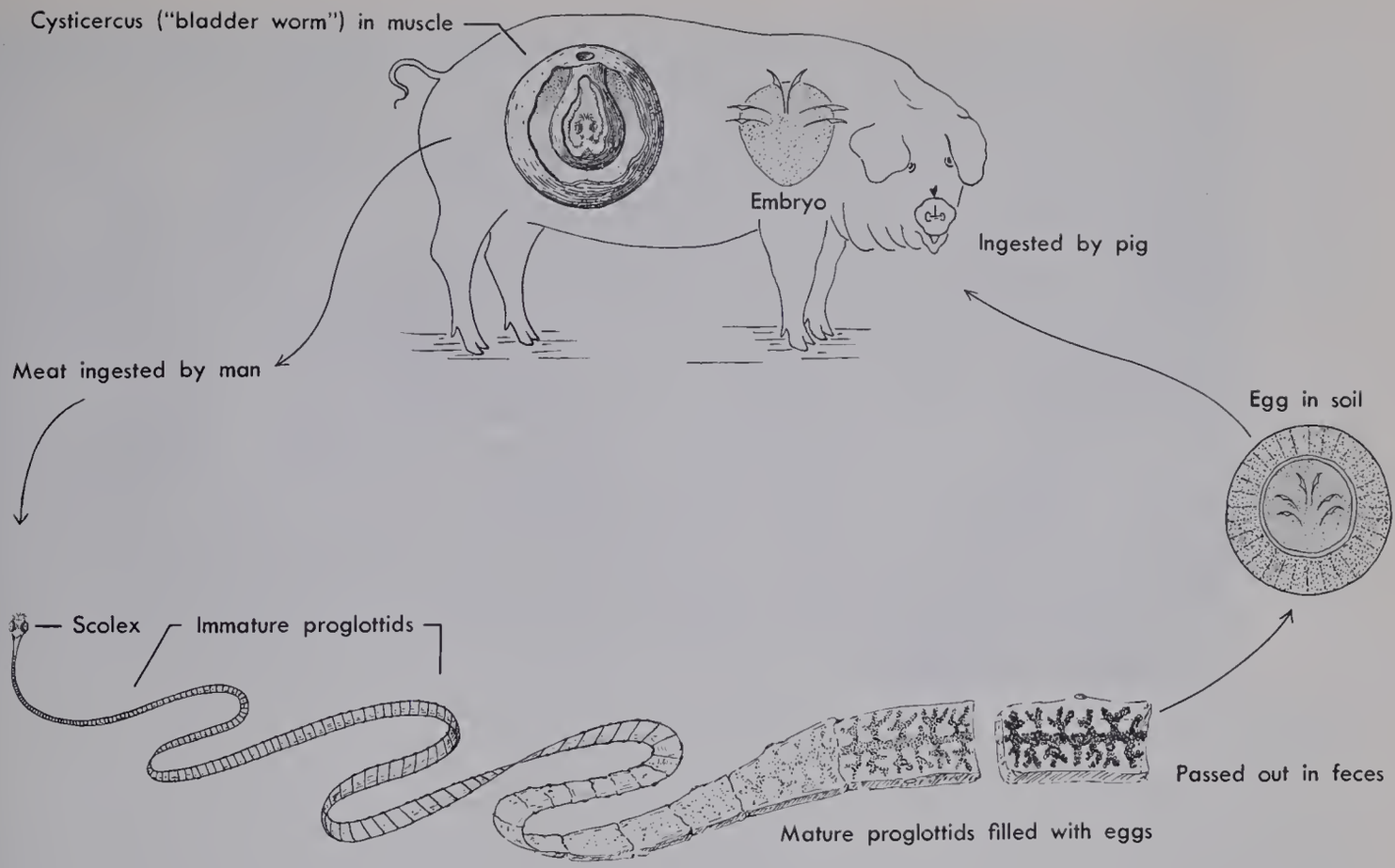


Fig. 16.2. Life cycle of the pig tapeworm *Taenia solium*. Asexual reproduction of proglottids by the scolex may produce a chain 20 ft long containing over 1000 proglottids. Each proglottid can be considered a separate individual. The proglottids reproduce sexually.

Budding in unicellular organisms

Asexual reproduction in yeast cells is by *budding*. Budding differs from fission in that the two parts produced are not of equal size. In the yeast cell, a bulbous projection, the bud, appears at one portion of the cell wall (Fig. 16.1). The nucleus of the parent cell divides and one of the daughter nuclei passes into the bud. Under favorable conditions, the bud may in turn produce another bud before finally separating from the parent cell.

16.2 ASEXUAL REPRODUCTION IN MULTICELLULAR ORGANISMS

1. Budding

Budding in animals

The term budding is also used to describe asexual reproduction in certain multicellular organisms. Undercooked pork may contain living "bladder worms" of the pig tapeworm, *Taenia solium*. These consist of a capsule containing a scolex (Fig. 16.2). When man ingests one of these, his gastric juice dissolves the wall of the capsule. The scolex turns inside out and attaches itself by suckers and hooks to the wall of the intestine. It then proceeds to produce buds, called **proglottids**, at its posterior end. These

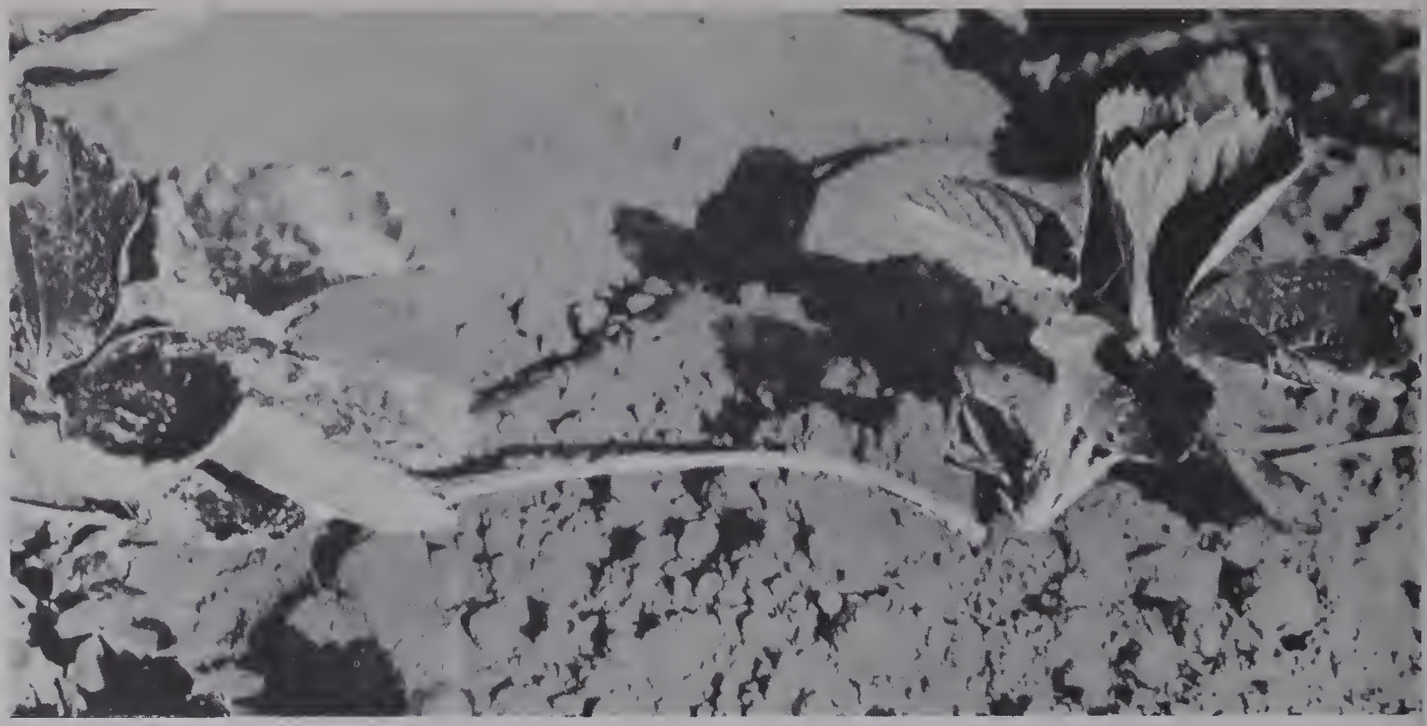


Fig. 16.3 Asexual reproduction in the strawberry. The daughter plants form along the horizontal stems (stolons).

remain attached to one another. As they mature, they develop organs for sexual reproduction. The most mature proglottids eventually break loose and are passed out in the feces. Before this happens, the chain may reach a length of 20 feet and contain over 1000 proglottids. Although there are rudimentary nervous, excretory, and muscular structures shared by the proglottids, each proglottid can be considered a separate individual.

Budding in plants

Similar *vegetative* methods of reproduction are found in plants. In some species, horizontal stems form which give rise to new individuals. These stems may extend underground (*rhizomes*) or along the surface of the ground (*stolons*) (Fig. 16.3). The houseplant *Bryophyllum* uses its leaves as organs of asexual reproduction. Along the margins, miniature copies of the plant form, complete with roots and stem (Fig. 16.4).



Fig. 16.4 Leaves of the common ornamental plant *Bryophyllum*. Note the miniature plants that have formed by asexual reproduction along their margins.

2. Sporulation

Spores are agents of dispersal

Fungi and certain plants carry out asexual reproduction by the formation of spores. These are small bodies containing a nucleus and a small amount of cytoplasm. The spores of terrestrial organisms are usually very light and have a protective wall around them. These two features make sporulation more than just a special reproductory mechanism. The small size and light weight of spores enable them to be carried great distances by air currents. Spores thus serve as agents of dispersal, spreading the organisms to new locations.

Spores may ensure survival during unfavorable environmental conditions

The resistant coat of the spore often serves another useful function. It enables the species to be maintained in a protected and dormant state over periods of unfavorable conditions which would be fatal to the active, vegetative organism. It is not surprising that this type of spore is produced most rapidly when conditions of temperature, moisture or food supply become less favorable.

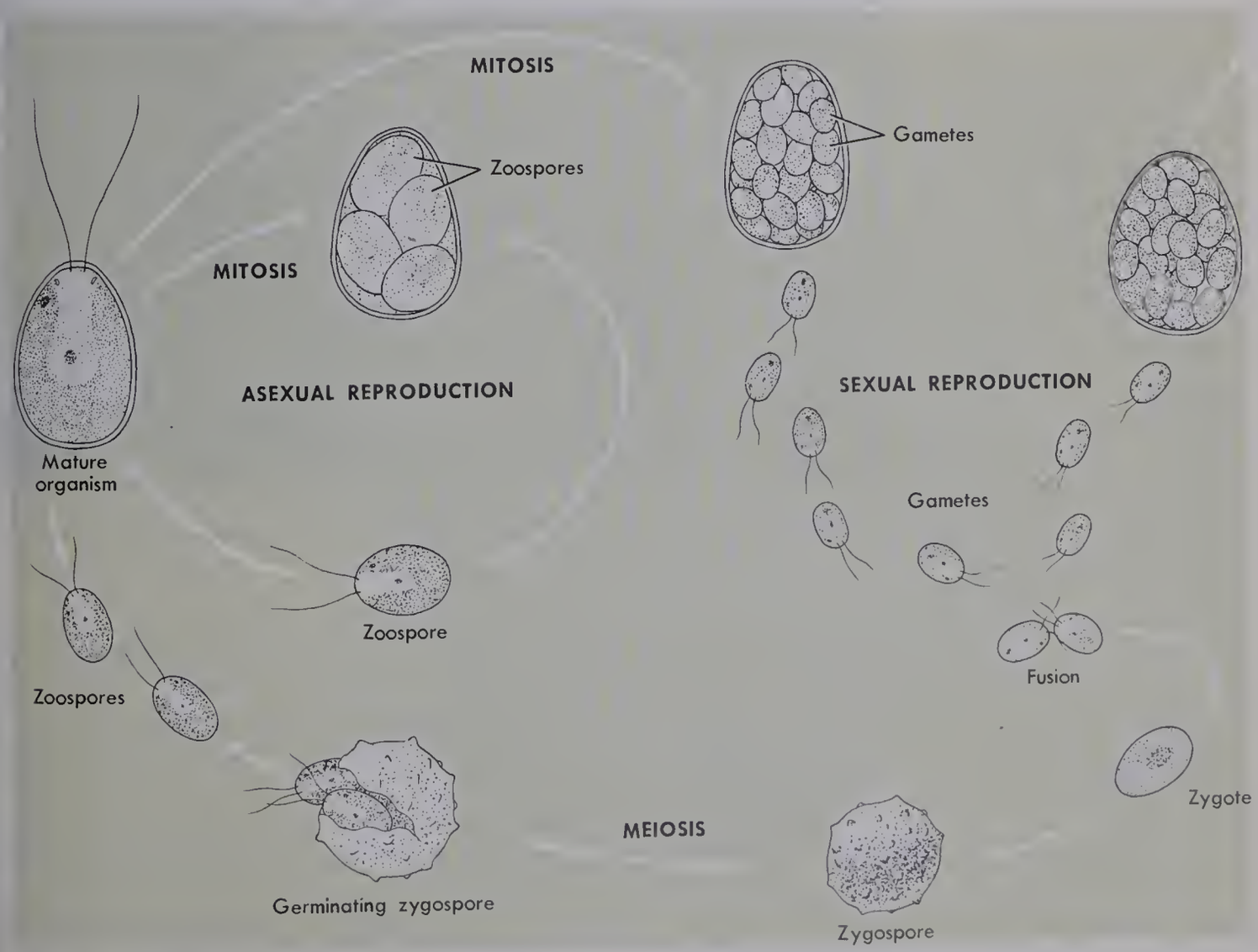


Fig. 16.5 Life cycle of *Chlamydomonas.* Although all its gametes look alike, two gametes from the same parent cell will not fuse with each other.



Fig. 16.6 Puffballs of the fungus *Lycoperdon.* Spores are produced asexually within the puffballs. (Courtesy of Jack Dermid.)

Many bacteria are spore formers. This cannot be considered a reproductory mechanism for them, however, for each cell simply forms a protective case around its "nucleus" and part of its cytoplasm. No new individuals are produced. The spore merely provides a means of survival during unfavorable conditions.

Zoospores

In some of the green algae and aquatic fungi, the spores are not resting stages. In *Chlamydomonas,* the contents of a single cell divide one to three times, forming two to eight small **zoospores,** each with its nucleus, cytoplasm, and two flagella (Fig. 16.5). After being released each zoospore grows to the full size of the parent cell. Some nonmotile algae use zoospores not only for reproduction but as a method of dispersal. Swimming by means of their flagella, the spores disperse the species to new locations.

The fungi are prolific spore producers. A single *Lycoperdon* mycelium produces approximately 700,000,000,000 spores in one season in its puffballs (Fig. 16.6). Spores of the wheat rust fungus have been recovered by aircraft flying 14,000 feet above the earth. If one leaves a piece of moistened bread (which does not contain a mold inhibitor) in a warm, dark place exposed to air currents, a luxurious growth of mycelium soon reveals how widely distributed fungus spores are (Fig. 16.7). The mosses, club mosses, and our common ferns also produce enormous numbers of tiny, windblown spores which serve to disperse the species to new locations.

3. Fragmentation

Fragmentation requires that the whole can be regenerated from a part

Some plants and animals accomplish asexual reproduction by fragmentation. In these species, the body of the organism breaks up into several parts. Each of these can then regenerate all the structures of the mature organism. In some tiny worms, the process occurs spontaneously. After the worm grows to full size, it breaks up into eight or nine pieces. Each of these then develops into a mature worm and repeats the process.

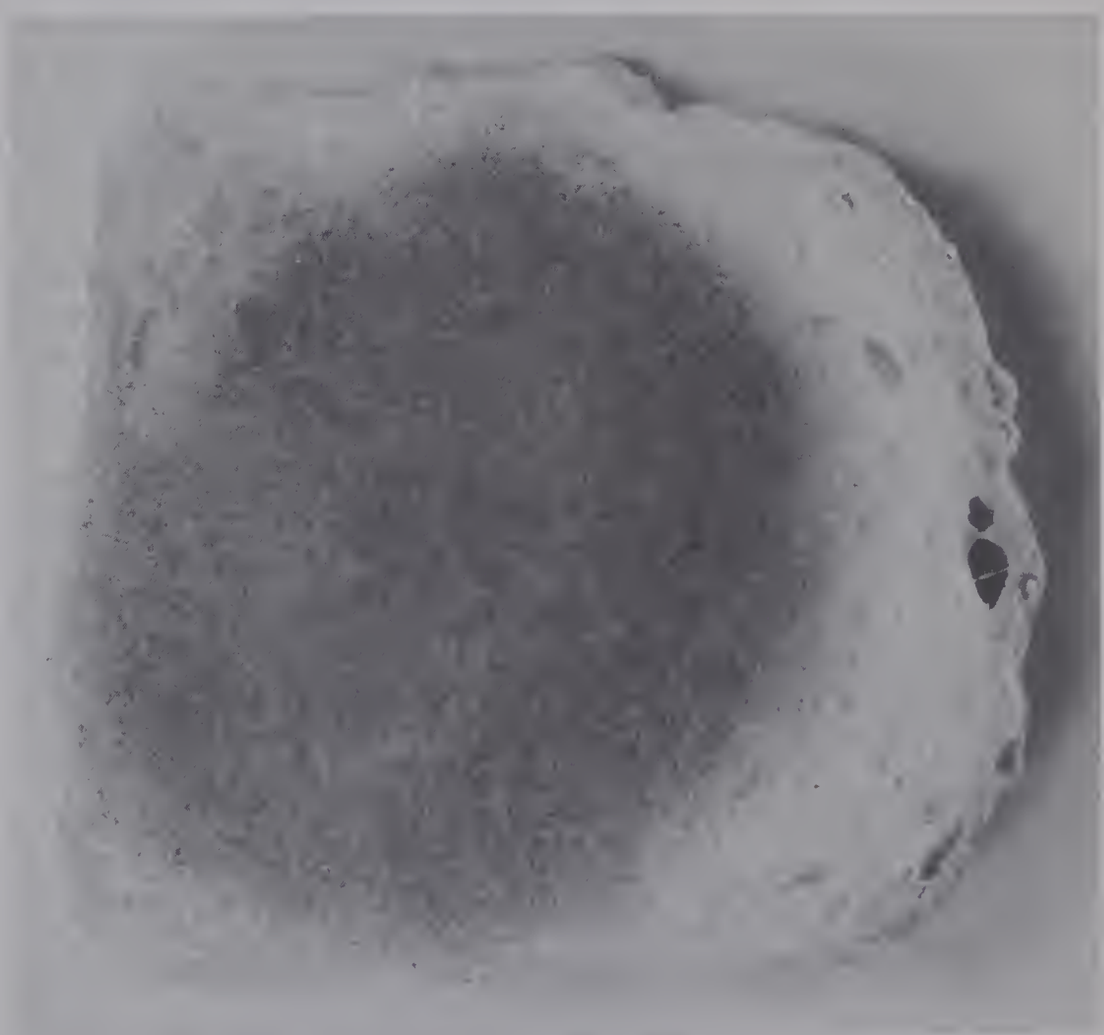


Fig. 16.7 This slice of bread was exposed to the air in a dusty room and then placed in a warm, moist, dark location. The mold is *Rhizopus nigricans.*

Most cases of fragmentation depend upon outside forces. Green and brown algae at the seashore are often broken into pieces by the action of the waves. Each of these pieces can then grow to full size. Even in fresh water, filaments of algae are frequently broken apart. Fission of the cells remaining in a fragment quickly rebuilds the filament.

Man has learned to fragment plants deliberately in order to reproduce desired varieties asexually. This is often done by making *cuttings* (see Fig. 24.16). With proper care, these develop roots and leaves and can take up an independent existence.

16.3 THE NATURE OF ASEXUAL REPRODUCTION

Grafting

Grafting is another asexual method of reproduction, which man uses to produce additional individuals of a desired variety. Only nurserymen deliberately grow apple trees by planting apple seeds. They do not raise these seedlings for the fruit they could produce but simply for a strong root system. After a year's growth, the portion of the plant above ground is removed and a twig (the **scion**) taken from a mature tree of the desired variety is inserted in a notch in the cut stump (the **stock**) (Fig. 16.8). So long as the cambiums of scion and stock are united and precautions are taken to prevent infection and drying out, the scion will grow. It will get all its water and minerals from the root system of the stock. However, the fruit that will be eventually produced will be identical (assuming it is raised under similar environmental conditions) to the fruit of the tree from which the scion was taken.

The need to specify similar environments is nicely illustrated in the wine industry. Most French vineyards are planted to grapes propagated vegetatively from California vines. However, the grapes of France (and the wines produced from them) are somewhat different from those of California.

Many cultivated plants are propagated asexually

The McIntosh apple is one of many popular varieties of apple grown in the United States and Canada. The first McIntosh tree was found over 150 years ago growing on the farm of John McIntosh in Ontario, Canada. It had grown from a seed. McIntosh's daughter-in-law appreciated the desirable qualities of the fruit. Moreover, she knew that it would be useless to try to grow other trees of the same kind from the seeds of the apples growing on this particular tree. Seeds, as we shall see in Chapter 20, develop as a result of sexual reproduction. Two parents are involved, and while one would be the McIntosh tree, the other parent would probably be some other apple tree growing in the vicinity. The offspring would possess characteristics of both parents. Perhaps it might produce better apples, perhaps worse. But it would not be a McIntosh. The only way in which the McIntosh could be made available to other apple growers was by *asexual* reproduction. Scions removed from the original tree and grafted onto stocks of any apple variety would produce McIntosh apples. Every one of the hundreds of thousands of McIntosh apple trees growing today is descended from a scion of that first tree. Or, to put it another way, all

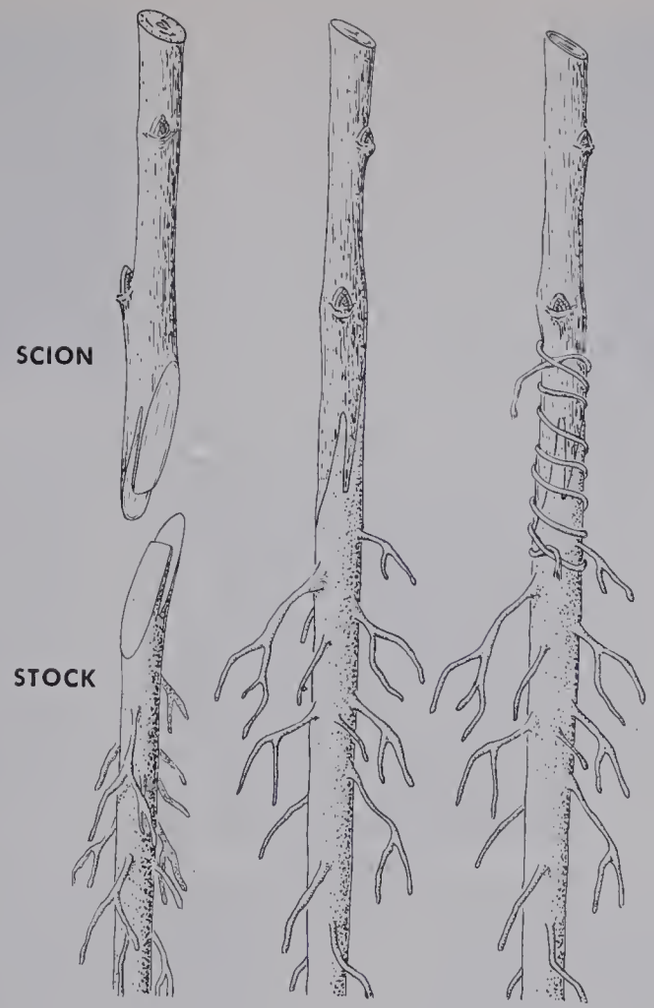


Fig. 16.8 Apple trees are propagated asexually by grafting. A piece of stem (the scion) from the desired variety is inserted into a notch cut in the stump of the stock. The fruit eventually produced will be characteristic of the variety that supplied the scion, not the variety that supplied the stock.

Clones are established and maintained by asexual reproduction

these trees make up a **clone.** They all share an identical genetic heritage because each has been produced by the continued division of the cells of that first tree.

This example of man-controlled asexual reproduction is useful because it points out the essential feature of this method of reproduction. In all kinds of asexual reproduction, the offspring are identical in every way to the parent so long as they are raised under environmental conditions similar to those of the parents. If a given species is successful in its habitat, any inheritable variation in the offspring may be disadvantageous. Asexual reproduction is a method for producing new individuals which will probably not show any such variations. It tends to preserve the *status quo.*

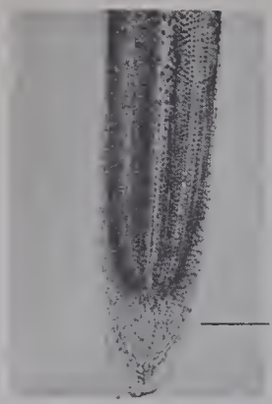
Asexual reproduction tends to preserve the status quo

The maintenance of clones depends on the accuracy of mitosis

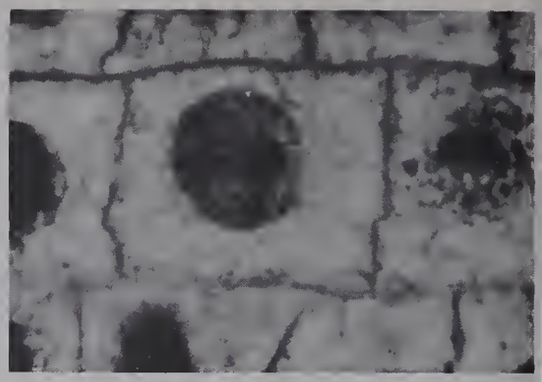
In all forms of asexual reproduction, new cells are produced by old. As our example of the McIntosh tree has shown, these new cells retain the same hereditary blueprints as their parents. In Chapter 5 (Section 5.4) we saw how Boveri's experiment showed that these hereditary blueprints reside in the nucleus. What in the nucleus contains them? When a cell divides, how is the information contained in the hereditary blueprints transmitted to its two daughter cells? As we shall see in Chapter 18, the answer to the first question is the *chromosomes.* The answer to the second question is *mitosis.*

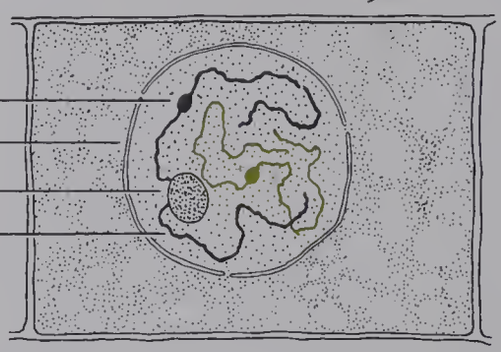


Onion roots

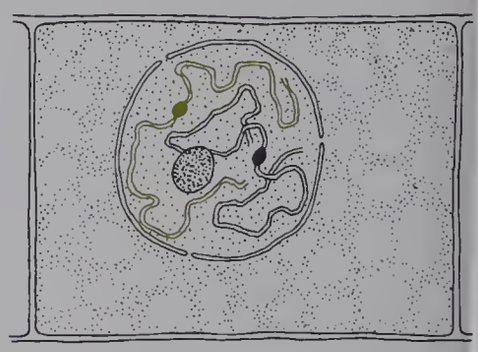


Single onion root showing meristem

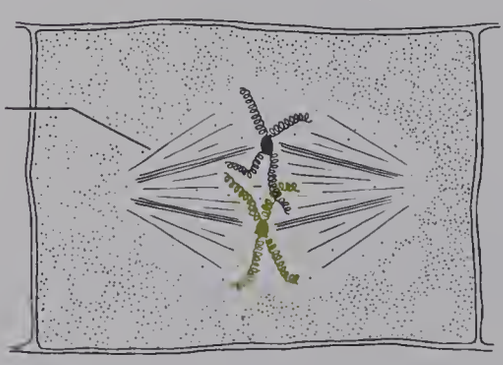




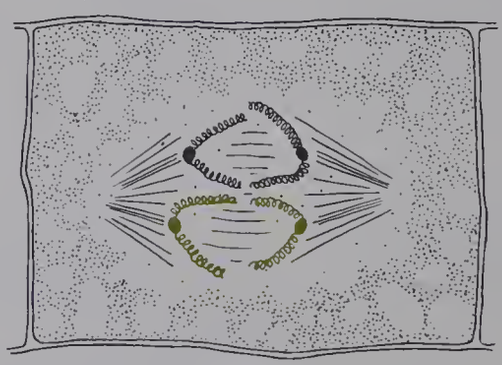
INTERPHASE
(before chromosome duplication)



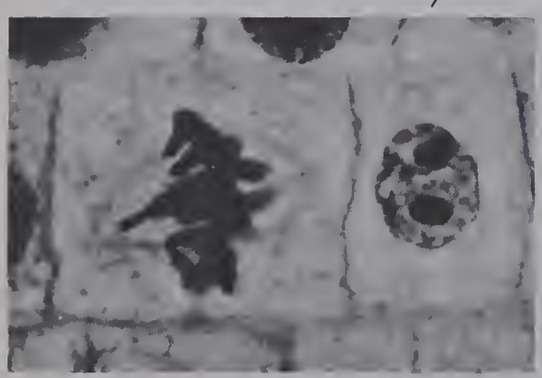
INTERPHASE
(after chromosome duplication)



METAPHASE



ANAPHASE





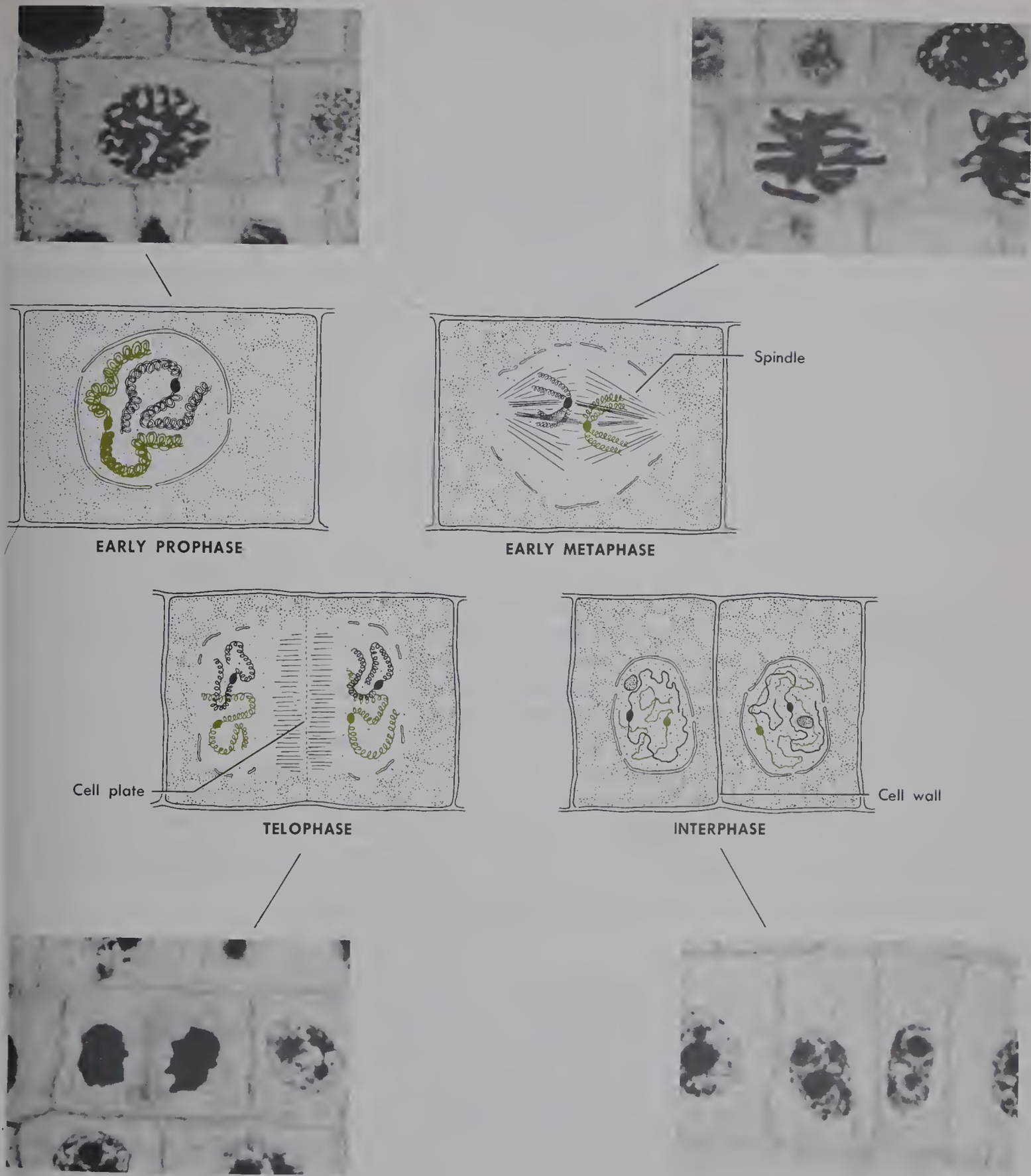


Fig. 16.9 Mitosis in a plant. The photographs show the stages as they occur in the dividing cells of the onion root tip. (Courtesy of the Carolina Biological Supply Co.) The drawings show the stages in a semidiagrammatic fashion. For the sake of clarity, only one pair of homologous chromosomes is shown: one member in black, the other in color.

16.4 MITOSIS

A convenient tissue in which to study mitosis is the meristem at the growing point of an onion root. Staining with an appropriate dye makes the chromosomes in dividing cells visible (Fig. 16.9).

Homologous chromosomes

A newly formed onion root cell contains 16 chromosomes; 8 of these had originally been contributed by the "father" of the onion plant, that is, the plant that supplied the male gamete. These chromosomes are often called *paternal* chromosomes. The other 8 were originally supplied by the onion's "mother," that is, the onion that produced the egg. These are the *maternal* chromosomes. For each of the maternal chromosomes, there is a paternal chromosome which looks just like it. These similar chromosomes make up a **homologous pair.** Each member of a given homologous pair is often referred to as the *homologue* of the other member of the pair.

When a cell is not actually in the process of dividing, the chromosomes (which are housed within the nucleus) cannot be seen under the light microscope. They are too tenuous to absorb very much stain and reveal their true nature. When the chromosomes are in this condition, they

Chromatin

are sometimes referred to collectively as the *chromatin* of the nucleus. In many cells, including the onion, one or more of the chromosomes has a nucleolus attached to it. This *can* be easily observed under the light microscope. The tenuous state of the chromosomes during the period between

The DNA content of the cell doubles between mitotic divisions

cell divisions should not suggest that they are inert at this time. Far from it. They are quite active in RNA synthesis and, at some time before the next cell division, DNA synthesis as well. In fact, chemical analysis shows that the DNA content is exactly *doubled.*

The phases of mitosis

The various events which occur during mitosis have been divided into four consecutive phases: prophase, metaphase, anaphase and telophase. The period between divisions is called interphase. It is important to realize that these phases are simply a convenient way of describing mitosis. The actual process involves (with a few exceptions) an unbroken sequence of events which merge smoothly with one another. The photomicrographs in Fig. 16.9 are "snapshots" of these various phases. Motion pictures would impart a better appreciation of the process.

Prophase. The onset of mitosis is marked by several changes. The nucleoli begin to disappear while the chromosomes themselves begin to appear. The previously extended strands of the chromosomes coil up into a helix, that is, like a cylindrical spring (Fig. 16.9). In so doing, they become shorter and thicker and thus more easily visible. At this time, the nuclear membrane begins to disappear.

Chromosomes are duplicated during interphase

The significance of the doubling of the DNA content of the cell before mitosis now becomes apparent. Each of the 16 chromosomes (8 homologous pairs) that was present in the cell when it was first formed now reappears, *doubled* (Fig. 16.9). The duplicates are attached to each other at a constricted region called the **centromere.** Although the term is not a technical one, we can refer to these duplicated chromosomes as making up a *doublet* (Fig. 16.10). The more common practice is to call the entire

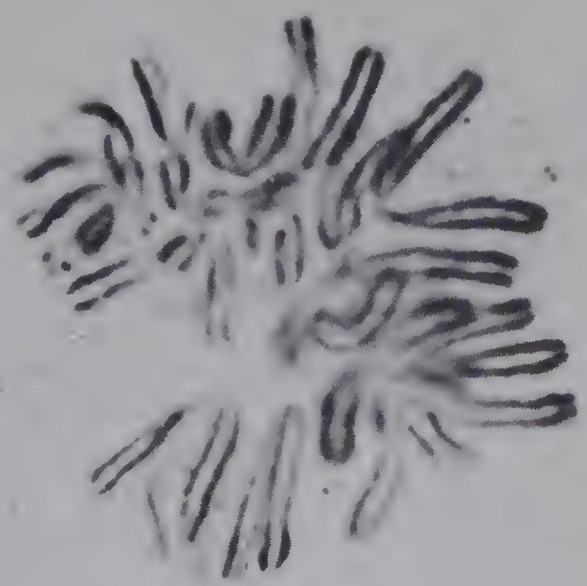


Fig. 16.10 Chromosomes in a dividing epidermal cell of a salamander. The duplicated chromosomes are still attached to each other by centromeres. (Courtesy of the General Biological Supply House, Inc.)

structure a chromosome and each of its strands a *chromatid.* This terminology is not so satisfactory because it obscures the fact that each member of a doublet is a *complete* chromosome equivalent in every way to the chromosome that the cell received when it was formed by a prior mitosis. In this sense, the onion cell in prophase has not 16 but 32 chromosomes consisting of 8 *pairs* of homologous *doublets.*

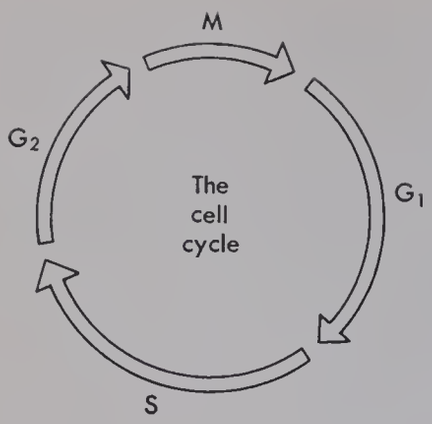
The spindle is an array of microtubules

Metaphase. Metaphase is marked by the appearance of the **spindle.** This structure consists of an array of microtubules (see Section 5.16) which extend between the ends or "poles" of the cell. The centromere of each doublet becomes attached to a microtubule and migrates to a point midway between the poles. The loose ends of the chromosomes may be oriented randomly, but all the centromeres lie exactly in a plane called the **equatorial plate** (Fig. 16.9).

The duplicated chromosomes separate in anaphase

Anaphase. Anaphase begins when the duplicated chromosomes (of the doublet) separate from one another. They now move apart, still on the spindle, and migrate to opposite poles, dragging their free ends behind them. The metaphor seems especially apt because the free ends of the chromosomes now turn toward the equatorial plate just as though friction with the surrounding cytoplasm were impeding their motion toward the poles.

Telophase. Telophase is roughly the reverse of prophase. Once the chromosomes reach their poles, they begin to uncoil. The nucleoli reappear. A nuclear membrane begins to form around the chromosomes. Finally, a structure called the **cell plate** appears at the equator. A cell wall is secreted on each side of the cell plate and cell division is thus completed.



Mitosis in animal cells

Interphase. Cytologists find it convenient to distinguish three periods during interphase. Once mitosis is complete, the cell begins a period of growth (called G_1). This is followed by a period (S) of DNA synthesis during which the chromosomes are duplicated. Then a second growth period (G_2) occurs prior to the next mitosis (M). These four periods in the life of dividing cells make up the "cell cycle."

Mitosis, as described above, is found universally among the plants. Animal cells, too, divide by mitosis. The phases are similar to those in plant cells, and the behavior of the chromosomes is the same. Two striking differences can, however, be observed. One is the appearance of the **asters.** Animal cells contain centrioles (see Section 5.17), and in prophase these migrate to the opposite sides of the nucleus. Here they seem to organize the formation of the spindle. Around each centriole also develops a system of radiating fibers, the aster (Fig. 16.11). The function of the aster is unknown. The second difference is that no cell plate is formed in animal-cell mitosis. Instead, in telophase, a furrow appears in the cell membrane, forming at the equator. The furrow deepens, and the two daughter cells simply become pinched apart. This process depends upon the presence of a belt of microfilaments (see Section 5.15) that extends around the cell and appears to provide the contractile force. The cell plate in plants is probably a consequence of the rigid cell wall which prevents furrowing from taking place.

How long does mitosis take? The entire sequence of phases may be completed in from nine minutes to many hours. The exact length of time varies considerably with different kinds of cells, different species of organism, and temperature.

What is the significance of mitosis? The most dramatic activity in mitosis is the organized behavior of the chromosomes. In fact, this aspect

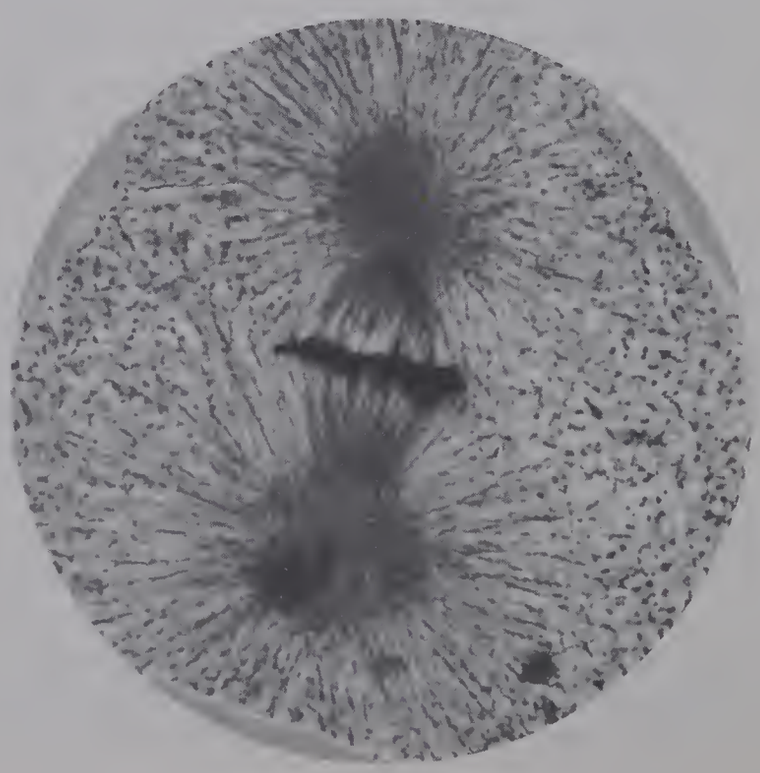


Fig. 16.11 Metaphase in a cell of the embryo of a whitefish. Note the prominent aster at each pole of the cell. (Courtesy of the General Biological Supply House, Inc.)

Significance of mitosis

of the process is so important that we still use the term mitosis even when the chromosomal activity occurs without actual division of the cell. Nuclear division without cell division occurs under certain conditions in a variety of organisms such as in the production of zoospores by *Chlamydomonas* (Fig. 16.5).

We know that the nucleus contains the information that controls the development and activities of the cell. The evidence of mitosis suggests that the chromosomes play an important part in this. Viewed in this light, mitosis can be considered a device for the orderly duplication (in interphase) and separation (in anaphase) of the chromosomes and thus of the hereditary information. Each daughter cell gets a complete set of chromosomes identical to those of the mother cell. Mitosis thus provides a way of transmitting this information unchanged and undiminished from parent cells to daughter cells.

How is mitosis used by living things? All the kinds of asexual reproduction discussed earlier are carried out by mitosis. In addition, growth, regeneration, and cell replacement in multicellular organisms are all accomplished by mitosis of the cells concerned.

SEXUAL REPRODUCTION

16.5 THE NATURE OF SEXUAL REPRODUCTION

In sexual reproduction, new individuals arise from the union of two sets of hereditary information (DNA). Usually, each of these sets is contained within a specialized cell called a **gamete.** In order to combine their hereditary information, the two gametes must first unite, a process known as fertilization. Among some simpler organisms such as the green alga, *Chlamydomonas,* the two gametes are indistinguishable from each other. Such similar-appearing gametes are called **isogametes.**

Isogametes

The nucleus and cytoplasm of a single *Chlamydomonas* cell divide five or six times to form 16 or 32 isogametes. These are simply miniature copies of the adult. The cell wall of the adult breaks open and releases the flagellated gametes into the surrounding water. These then fuse with the gametes produced by other *Chlamydomonas* cells. There are no visible differences between the fusing gametes (Fig. 16.5).

In most organisms, two quite distinct kinds of gametes are produced. **Sperm** are tiny gametes which consist of little more than a nucleus and a flagellum. The flagellum enables the sperm to swim. Sperm are the male (♂) gametes. **Eggs,** the female (♀) gametes, are larger and nonmotile. They contain substantial quantities of food in addition to the nucleus. Because sperm and eggs are so dissimilar in appearance, they are called **heterogametes.**

Heterogametes

The product of fertilization is the **zygote.** The zygote resulting from the union of heterogametes is frequently referred to as a fertilized egg.

One of the most striking features of sexual reproduction is that each of the gametes involved is usually produced by a separate individual. Thus, two individuals, the parents, contribute to the formation of the offspring. If the two parents differ in any way (and often, as you will see, even if they don't), the offspring will possess new combinations of characteristics. Many times these new characteristics may be disadvantageous to the offspring. Many times they will have no effect on the welfare of the possessor. Sometimes the new combination will result in an individual better suited than its parents to the habitat in which it lives. It is this latter category that permits gradual structural and functional improvements in a species, that is, evolution.

Sexual reproduction provides new combinations of traits

In many organisms, both male and female gametes are produced in one individual. Such organisms are said to be hermaphroditic. The common earthworm, some fishes and the majority of our flowering plants are examples of hermaphroditic organisms. Even in these cases, two individuals usually contribute to the formation of the zygote. Sperm cells from one individual unite with the eggs of another individual. **Cross-fertilization** is the result, and variability in the offspring is thus promoted. Some hermaphrodites, such as the earthworm, never fertilize their own eggs. Others, such as many of the flowering plants, engage in self-fertilization only when cross-fertilization fails to occur. In Chapter 20 we shall examine some of the mechanisms by which cross-fertilization in plants is promoted and self-fertilization is avoided.

Hermaphrodites

16.6 SEXUAL REPRODUCTION IN BACTERIA

The essence of sexual reproduction, then, is newness, change, variability. This is perhaps best illustrated by the process as it occurs in some bacteria. For many years, bacteria were believed to reproduce by fission only. No evidence of sexual reproduction in them was detected until 1947. In that year, J. Lederberg and E. L. Tatum discovered that the common intestinal bacterium *Escherichia coli* could reproduce sexually. Although the cells were not actually observed in the process for some time after, Lederberg and Tatum did find definite, if indirect, evidence that sexual reproduction was taking place.

"Wild-type" E. coli *can live on glucose and salts*

The particular strain of *E. coli* that they used is not at all fussy about its food supply. Given glucose as a source of energy and carbon, and some inorganic salts to supply nitrogen, sulfur, and phosphorus atoms for the synthesis of amino acids and nucleic acids, the organism grows well. Within its tiny cell, it contains all the enzymes with which to carry out the biochemical reactions needed to convert glucose and inorganic salts into all the organic molecules necessary to life.

E. coli, like all bacteria, is quite sensitive to exposure to ultraviolet light. A moderately heavy dose is lethal. In fact, this is why ultraviolet

Ultraviolet light is used to produce nutritionally-deficient strains

lamps are sold as "germicidal" lamps to be used for killing bacteria in hospital operating rooms, etc. Lesser exposures to ultraviolet light may not kill the bacterium outright but, instead, destroy its ability to manufacture one or more important organic compounds. These compounds must then be added to the bacterium's diet if it is to survive. Furthermore, this loss of synthetic ability is hereditary. All the offspring produced by the fission of a *nutritionally deficient* bacterium will suffer the same disability.

A strain of *E. coli* has been developed by ultraviolet irradiation that lacks the ability to synthesize one of the B-vitamins, biotin, and one of the amino acids, methionine. These two substances must be added to its diet in order for it to live. Another strain has been developed that can still synthesize biotin and methionine from glucose and salts but is incapable of synthesizing another B-vitamin, thiamin, and the two amino acids, threonine and leucine.

When two complementary nutritionally-deficient strains are cultured together, wild-type bacteria reappear

If these two strains of bacteria are allowed to mingle freely with each other and then placed on a culture medium containing only glucose and salts, colonies of cells soon appear. These cells thrive despite the absence of the vitamins and amino acids needed by their parents. Furthermore, descendants of these cells continue to be able to live on glucose and salts alone.

What has happened? Evidently, members of each strain have in some way managed to combine their hereditary blueprints. The first strain with blueprints for making the enzymes needed to synthesize thiamin, threonine, and leucine (as well as all the other organic molecules except biotin and methionine) has combined these blueprints with those of the second strain which included the instructions for making biotin and methionine. Thus, a bacterium was produced which, like the original strain, is capable of satisfying all its needs from glucose and salts alone. The evidence indicates that a bacterium of one strain actually injects part of its single chromosome into a cell of the other strain when the two cells come in contact.

Note that in this example, sexual reproduction has been reduced to its essence. No gametes are formed; one cell simply attaches temporarily to another and donates some of its hereditary blueprints to it. Nor have *additional* bacteria been created by this process. A *new* bacterium has, though, a bacterium capable of living under conditions neither of the parental types could survive in.

A problem remains. Sexual reproduction in bacteria involves the transfer of a piece of chromosome from one cell to another. It is not reciprocal: that is, no exchange is involved. Thus one cell acquires a greater total hereditary blueprint. This must be followed sometime by the elimination of an equivalent amount. Otherwise, over a period of time, there would be a steady accumulation of chromosomal material in the species. It is not yet certain by just what mechanism a bacterial cell reestablishes its normal chromosome content. The same problem faces other sexually reproducing organisms, however, and its solution in them *has* been discovered.

16.7 MEIOSIS

The diploid number of chromosomes

Each species of organism has a characteristic number of chromosomes. The little fly *Drosophila melanogaster* has 8, the onion 16, and man has 46. (Lest you assume from this that the number of chromosomes in a species is necessarily a function of the complexity of the organism, you should note that the crayfish has at least 200 chromosomes in its cells.) All these numbers are even. This reflects that fact that the chromosomes are present in homologous *pairs.* Cells containing a full complement of homologous pairs are said to contain the **diploid** or $2n$ number of chromosomes.

Mitosis results in the formation of cells that have exactly the same number of chromosomes as the parent cell. This would create difficulties if the cells being formed were to serve as gametes. A human sperm with 46 chromosomes uniting with an egg containing 46 chromosomes would result in a zygote with 92 chromosomes—twice the normal number for the species. Development of the zygote by mitosis would result in all subsequent cells having the new number. You can readily see that after a few generations of this, there would be no room in the cell for anything but chromosomes.

Actually, this situation rarely arises in living things. At some point between the formation of the zygote and the formation of gametes, a special kind of cell division occurs, called **meiosis.** Meiosis consists of two consecutive cell divisions with but one duplication of the chromosomes.

Meiosis halves the number of chromosomes

When a cell divides by meiosis, then, four cells are produced, each containing just one-half the normal diploid or $2n$ number of chromosomes. This half-number is called the **haploid** or n number. The reduction in chromosome number is not a random process. In cells produced by meiosis, only one member of each of the homologous pairs of chromosomes of the diploid cell is present. Thus when two gametes unite, the resulting zygote ($2n$) gets one member of each pair of homologous chromosomes from each gamete and thus from each parent.

Each of the two meiotic divisions can be separated into phases similar to those occurring in mitosis. However, significant differences in the behavior of the chromosomes occur in the first of the divisions.

The first meiotic division

Prophase of the first meiotic division is a much slower and more complex process than in mitosis. The most striking event during this stage is the fusing together of each pair of homologous doublets, a process called **synapsis.** As they lie united lengthwise, two of the chromosomes (chromatids), one from each doublet, "cross over." In so doing, they *exchange* one or two sections (Fig. 16.12).

In metaphase, the centromeres of each homologous pair become attached to the spindle, one above, the other below the equatorial plate. With the onset of anaphase, each centromere moves toward its respective pole without duplicating. Thus telophase produces two cells, each of which has just one member of each homologous pair of chromosomes that was present in the original cell.

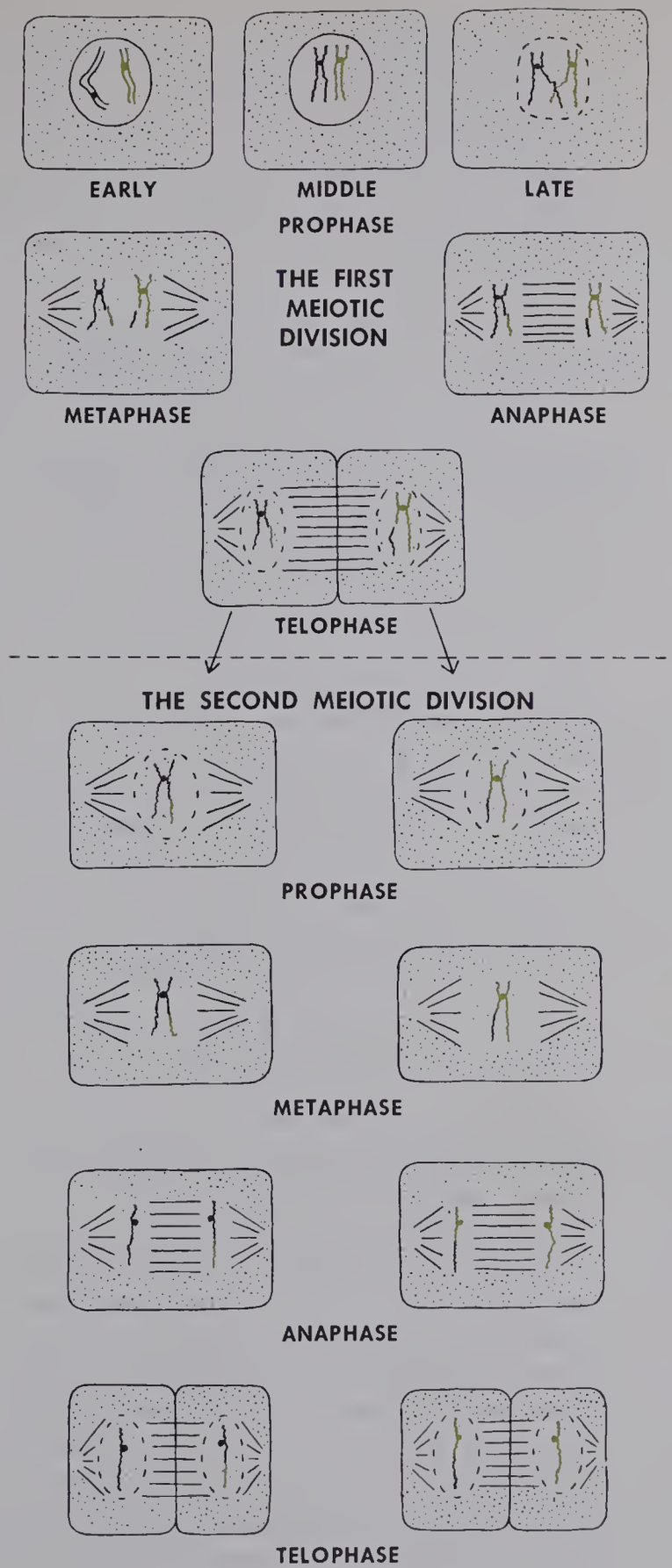


Fig. 16.12 Meiosis. The behavior of just a single pair of homologous chromosomes is shown with one homologue black, the other in color.

The second meiotic division

The second meiotic division occurs at varying lengths of time after the first division, depending on the species. It is similar to a mitotic division. The chromosomes are still present as "doublets." The two chromosomes of each doublet are not likely to be identical, however. A glance at Fig. 16.12 will show why this is so. In the first meiotic division, crossing over between the paternal and maternal homologues resulted in one chromosome (chromatid) of each doublet no longer being identical to the other chromosome of the doublet.

After moving to the equatorial plate, the chromosomes separate and each migrates to its respective pole (Fig. 16.12).

With the completion of the second meiotic division, a total of four cells has been produced. Each contains just one member of each homologous pair of chromosomes present in the original cell. These cells thus contain just one-half (the haploid number) of the chromosomes of the parent cell. Furthermore, in our simplified case, two of the four cells produced have an unchanged maternal or paternal chromosome; the others have a chromosome containing *both* maternal and paternal parts.

Crossing over is not the only way by which meiosis produces cells whose chromosome content differs. In Fig. 16.12, for the sake of simplicity, only one pair of chromosomes ($2n = 2$) is shown. In most living things, the diploid number is larger. The parasitic roundworm, *Ascaris bivalens,* has a diploid number of 4. These four chromosomes make up two homologous pairs. One member of each pair came from the worm's father, the other from its mother. Thus we can indicate the two pairs as A^mA^f and B^mB^f. When *Ascaris* undergoes the first meiotic division, the homologous pairs orient themselves on either side of the equatorial plate. If A^m and B^m orient on one side and A^f and B^f on the other, the gametes will be the same (neglecting crossing over) as those the worm received from its parents. It is just as likely, however, that A^m and B^f will orient on one side of the equatorial plate and A^f and B^m on the other (Fig. 16.13). The gametes produced by this arrangement will contain a new combination of chromosomes. Any organism whose diploid number is 4 can thus produce four kinds of gametes: A^mB^m, A^fB^f, A^mB^f, or A^fB^m. The fly *Drosophila melanogaster* has 8 chromosomes in its diploid cells, 4 derived from its mother and 4 derived from its father. In distributing one of each homologous pair to the gametes, 16 different combinations can be produced. The number of different kinds of gametes produced by this **random assortment** of the chromosomes is equal to 2^n where n = the haploid number of chromosomes in the organism. Human mothers and fathers, each with a haploid number of 23, can each produce by random assortment alone 2^{23} or 8,388,608 different kinds of gametes. When you consider further that crossing over will have occurred between their own paternal and maternal chromosomes in the first meiotic division, it is not surprising that no two people ever resemble each other exactly. (An exception to this is the special case of identical twins; but it is only an apparent exception since they arise from a *single* fertilized egg.)

Random assortment of the chromosomes occurs during meiosis



Fig. 16.13 Random assortment of maternal (black) and paternal (color) chromosomes during meiosis in *Ascaris bivalens* ($2n = 4$).

Significance of meiosis

What, then, is the significance of meiosis? First, the number of chromosomes is reduced from the diploid number to the haploid number. This sets the stage for the union of two gametes and thus provides a mechanism by which traits of two different parents can be combined. Second, meiosis provides for variation in the gametes produced by each parent. **Random assortment** of the paternal and maternal chromosomes *plus* the **crossing over** of portions of the maternal and paternal chromosomes make it likely that no two gametes of even one parent will be exactly alike.

Meiosis occurs at some time between fertilization and gamete formation

How is meiosis used by living things? You have seen the necessity for meiosis to occur sometime between fertilization and gamete formation in sexually reproducing forms. In animals, meiosis leads directly to the production of gametes. Among the fungi and algae, on the other hand, meiosis is apt to occur very soon after the formation of the zygote. The zygote formed by the fusion of two *Chlamydomonas* gametes develops a heavy wall and becomes dormant. When favorable conditions exist, the zygote undergoes meiosis, producing four haploid zoospores (Fig. 16.5). These are released and soon grow to full-size *Chlamydomonas* cells. Most of the life cycle of this organism is thus spent in the haploid condition. In plants, too, meiosis is used in the production of spores. Only later are gametes derived from these.

The occurrence of meiosis long before the formation of gametes in many organisms illustrates two additional points about cell division. First, *mitosis* can occur in haploid cells as well as diploid ones. Second, the haploid number of chromosomes is sufficient to control cell functions in *these* organisms (but not in many animals) and reestablishment of diploidy by fertilization simply provides a double set of the hereditary information.

This double set does, however, lay the basis for a reorganization of the hereditary information at the next meiosis. Once again, we see sexual reproduction serving one major function: the production of variability in the species.

Meiosis followed by fertilization generates the variability associated with sexual reproduction

Earlier in the chapter it was pointed out that asexual reproduction usually results in offspring that are exact copies of their parents. Sexual reproduction, on the other hand, was described as a method of producing offspring with characteristics different from those of the parents and thus of producing variability within the species. Assuming that the information that controls the development of the characteristics of an organism is located in its chromosomes, it is easy to see how mitosis makes the first possible and meiosis, followed by fertilization, makes the second possible.

Throughout this chapter, we have referred often to the hereditary blueprints within the nuclei of cells. One of the most active fields of biological research is that concerned with the nature and operation of these hereditary blueprints. This study is called genetics. It is the topic of the next three chapters.

EXERCISES AND PROBLEMS

1. The haploid number of horse chromosomes plus the haploid number of donkey chromosomes can produce a healthy mule. The mule is sterile. With rare exceptions (see Question 2), it cannot breed with either another mule or a horse or a donkey. Can you explain why in terms of the behavior of the chromosomes during meiosis?
2. Although mules are generally sterile, a few cases are known where a female mule has given birth to a horse (after mating with a horse) or another mule (after mating with a donkey). Using your knowledge of meiosis, can you think of an explanation for these rare events?
3. How does the behavior of the chromosomes in mitosis differ from their behavior in the first meiotic division?
4. Asexual reproduction preserves the *status quo.* Sexual reproduction promotes change. Describe how mitosis makes the first possible and meiosis followed by fertilization makes the second possible.
5. Explain why the minimal medium for growing *E. coli* contains (a) glucose or some other carbohydrate, (b) KH_2PO_4, (c) $MgSO_4$, (d) NH_4NO_3.
6. If a hermaphroditic organism fertilizes its own eggs, must all its offspring be identical? Explain.
7. How many kinds of gametes can the onion ($2n = 16$) produce by random assortment alone?
8. What functions do spores accomplish in the bread mold, *Rhizopus nigricans?*

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17 GENETICS: THE WORK OF MENDEL

The Theory of the Inheritance of Acquired Characteristics

Mendel's Theory: the Background

Mendel's Experiments

Mendel's Hypothesis

How Hypotheses are Judged

The Testcross: a Test of Mendel's Hypothesis

Dihybrids—the Law of Independent Assortment

Mendel's Theory: the Sequel

Continuous Variation: the Multiple-Factor Hypothesis

Genetics: determining the rules of inheritance

When living things reproduce asexually, their offspring develop into exact copies of their parents so long as they are raised under similar conditions. On the other hand, when living things reproduce sexually, their offspring develop traits different from one another and different from those of either parent. When a Collie and a German Shepherd mate, their offspring are dogs, not some other species of animal. Their offspring are, however, neither Collies nor German Shepherds. Long before biologists discovered many of the facts of mitosis and meiosis, they tried to discover rules which would explain how the observable characteristics of offspring are related to those of their parents and even their parent's parents.

Of the several theories that have been formulated to explain how traits are inherited, two deserve special mention. One of these, Mendel's theory, has provided the foundation upon which all later work in genetics has been built. The other, the theory of the inheritance of acquired characteristics, has failed to pass scientific tests but, despite this, has continued to have its defenders.

17.1 THE THEORY OF THE INHERITANCE OF ACQUIRED CHARACTERISTICS

Lamarck

This theory states simply that traits *acquired* by parents during their lifetime can be passed on to their offspring. The theory is usually associated with the name of Lamarck, a French biologist, who used it in an attempt to explain the many striking adaptations to their environment shown by plants and animals. His most famous illustration was the giraffe. He claimed that the long neck of the giraffe has evolved as a result of generations of giraffes stretching their necks to browse on the leaves of trees. Each generation has passed on to its offspring the small increase in neck length caused by continual stretching.

Evidence that weakens Lamarck's case

Is there any evidence that such a phenomenon occurs? Despite repeated attempts to prove that changes in the body acquired by an individual can be passed on to its offspring, no evidence for it has yet been discovered. The earliest experiments that attempted to settle the question were those in which some body part, such as the tail of a mouse, was removed surgically. Even after generations of tail removal, young mice were born with as long tails as ever. Actually, the experimenters could have simply looked about them for corroboration of their findings. Sheep raisers have been removing the tails of lambs through uncounted generations, but the process must still be carried out with each new generation. Even when more sophisticated attempts were made to alter heredity by altering the environment, it could not be done.

Two gametes constitute the narrow genetic bridge between one generation and the next

Why not? In order for changes carried out on the body to be transmitted to the offspring, they would have to become incorporated into the sperm and egg cells, the only link between the bodies of the parents and the bodies of the children. Perhaps such a thing might occur if the specialized cells of the body, upon which some alteration could be made, then produced the gametes. But they do not. For many years it has been known

that in animals the gamete-producing cells of the body are set aside very early in embryonic life. In fact, a newborn girl has already set aside and begun the first meiotic division for each and every cell that will someday develop into a mature egg.

Germplasm vs. somaplasm

The German biologist, Weismann, expressed these thoughts in the form of his theory of the *continuity of the germplasm.* Multicellular organisms, according to his theory, are made up of gamete-producing cells or **germplasm** and the cells of the rest of the body, the **somaplasm.** Weismann considered the germplasm immortal, an unbroken chain of gametes and embryos going right back to the dawn of life (Fig. 17.1). At each

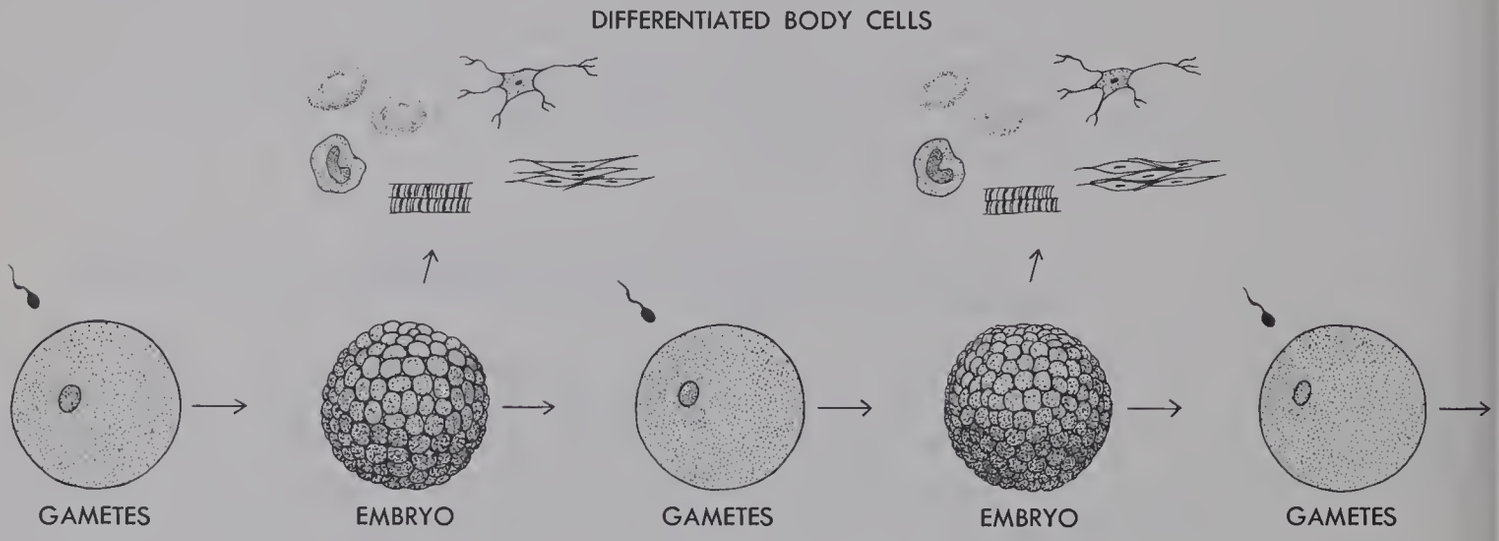


Fig. 17.1 Weismann's hypothesis. While true for most animals, it does not hold true for plants. Plants *can* produce gamete-forming cells from differentiated body cells.

generation, the embryo that develops from the zygote not only sets aside some germplasm for the next generation but also produces the cells that will develop into the body, the somaplasm, of the organism. In this view, the somaplasm simply provides the housing for the germplasm, seeing to it that the germplasm is protected, nourished, and conveyed to the germplasm of the opposite sex in order to create the next generation. The old riddle about which came first, the chicken or the egg, would have been no puzzle to Weismann. In his view, the chicken is simply one egg's device for laying another egg.

The essential truth of Weismann's theory was beautifully demonstrated in 1909 by the Americans W. E. Castle and John C. Phillips. They removed the ovaries from an albino guinea pig and substituted the ovaries from a black guinea pig. Then they mated this albino guinea pig with an albino male. Instead of getting albino babies as might normally be expected, the babies were black. (Matings between albino and black guinea pigs always produce black offspring.) The hereditary blueprints of the eggs had not been altered by their maturation in the body of a different animal.

Despite this and many other experiments refuting the idea that what happens to the somaplasm can affect the germplasm, some scientists still persist in trying to prove otherwise. This has been particularly true of those who were believers in Communism. They seem to feel that the physical and mental benefits that one generation acquires through its hard work *ought* to be transmitted to its offspring. There have even been cases where scientists falsified their experimental results in their eagerness to substantiate the theory that acquired traits can be inherited. Fortunately, one of the cornerstones of the scientific process is that if other workers cannot duplicate certain scientific findings, they will not be accepted as valid.

Let us now look at the theory which, having met test after test, is accepted by the vast majority of geneticists and forms the basis upon which most of the useful discoveries in genetics have been made.

17.2 MENDEL'S THEORY: THE BACKGROUND

Our present theories of inheritance were first worked out by the Austrian monk, Gregor Mendel (Fig. 17.2). From 1858 to 1866, Mendel worked in the garden of his monastery, in the town of Brünn (now Brno) breeding garden peas and examining the offspring of these matings. Mendel was not the first man to carry on controlled breeding experiments in an attempt to discover rules of inheritance. He succeeded where others had failed, however, because (1) he chose his experimental organism with care, (2) he limited the scope of his investigation, and (3) he recorded his data carefully and subjected them to mathematical analysis. Mendel's decision to work with the common garden pea was an excellent one. The plant is hardy and fast growing. As in many legumes, the petals of the flower entirely enclose the sex organs. These are the **stamens,** which produce pollen (the bearer of the male gametes) and the **pistil,** which produces the female gamete, the egg. Although insects may occasionally penetrate to the sex organs, self-fertilization is the rule. Mendel found he could open the buds and remove the stamens before they ripened. Then, by dusting the pistil with pollen from another plant, he could effect cross-fertilization between them.

Artificial pollination in the pea plant

The choice of the garden pea was a happy one also because many varieties existed which differed from one another in clear-cut ways. Some produced (after drying) wrinkled seeds. Others produced smooth, round seeds. Some produced seeds with green cotyledons; others with yellow cotyledons. Some produced green pods; others, yellow pods. Some produced white flowers; others reddish flowers. These paired characteristics (and three others) Mendel chose to study because they were so easily distinguishable and because they "bred true" generation after generation. That is, so long as they were maintained by normal self-pollination, these varieties continued to produce offspring identical to the parents in the characteristics being studied.

There were other characteristics in which Mendel's pea varieties differed, such as leaf size and flower size. Mendel wisely ignored these

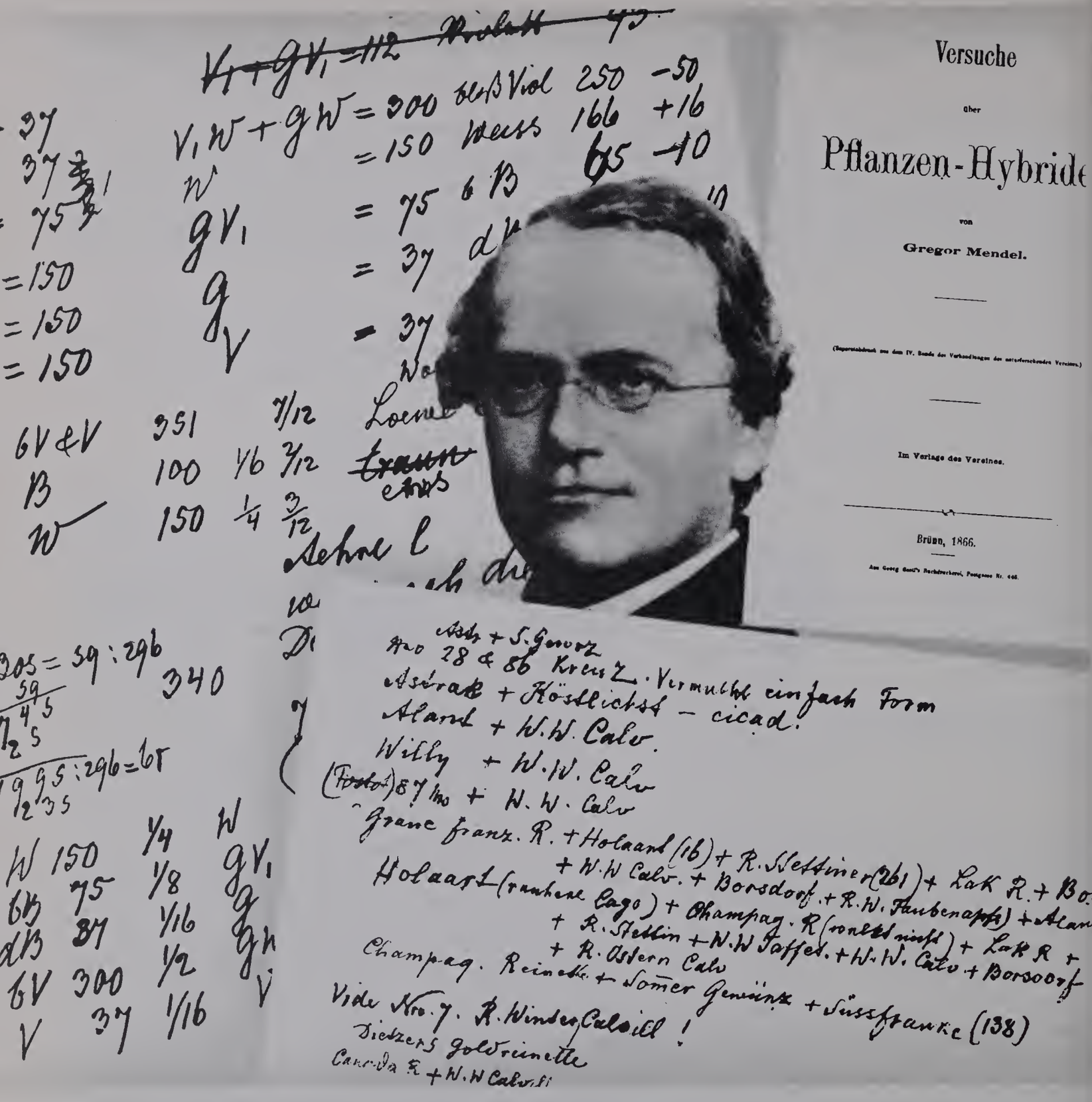


Fig. 17.2 Gregor Mendel (1822–1884), with the title page of the paper in which he published his work, and some of his notes on plant genetics and hybridization. (Photo courtesy of Professor Verne Grant.)

differences in his studies simply because they did not fall into a clear-cut "either-or" classification. Mendel's peas produced either round seeds or wrinkled seeds. There were no intermediate types. On the other hand, the size of leaves and flowers varied over quite a range. There were not just two distinct categories in which they could be placed. Mendel's decision to thus limit the scope of his experiments was certainly an important factor in his success.

Mendel studied traits that were sharply demarcated

Finally, Mendel recognized the need to keep careful records of the various kinds of offspring produced by his experimental breeding. After counting the different kinds produced, he subjected the figures to statistical analysis. This analysis led him, as we shall see, directly to his theory of inheritance.

17.3 MENDEL'S EXPERIMENTS

The P_1 generation

In one of his first experiments, Mendel crossbred a round-seeded variety with a wrinkle-seeded variety. This parental generation he called the P_1 generation. Pollen from the stamens of the round-seeded variety was dusted on the pistils of the wrinkle-seeded variety. The reciprocal cross was carried out: pollen from the stamens of the wrinkle-seeded variety was placed on the pistils of the round-seeded variety. In both cases, every one of the seeds produced by these cross-fertilized flowers was round. There were no seeds intermediate in shape. (Seed shape and cotyledon color were particularly rewarding characteristics to study. Their form could be determined in the same season that the fertilization was carried out. The seeds *were* the next generation. Pod shape, stem length, and flower color in the second generation could not be determined until the following season when the seeds had germinated and developed into the mature plants.)

The F_1 generation

Mendel called the second generation the **hybrid** generation because it was produced by dissimilar parents. It is also called the F_1 generation.

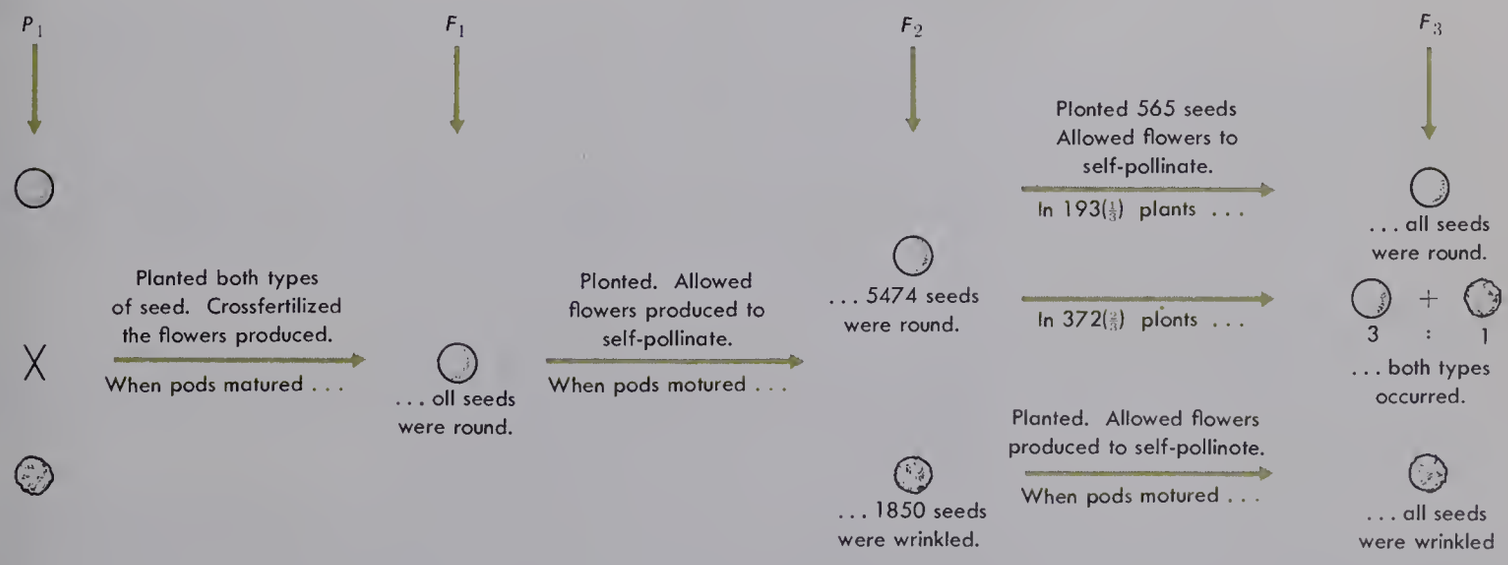


Fig. 17.3 Mendel's results.

The F_2 generation

Mendel planted all his F_1, round seeds; 253 F_1 plants grew to maturity. The F_1 flowers were allowed to self-fertilize themselves in the normal way. In effect, Mendel was breeding the F_1 (or hybrid) generations together. From the pods of these F_1 plants, Mendel recovered 7324 seeds, the F_2 generation. Of these, 5474 were round, 1850 wrinkled. Dividing the larger number by the smaller, a ratio of 2.96 round to 1 wrinkled is derived.

The F_3 generation

Mendel then planted some of these two kinds of F_2 seeds. From the wrinkled seeds, he raised plants which produced (by self-fertilization) a new crop (F_3) of seeds. These were exclusively of the wrinkled type. From the round seeds, he raised 565 plants and allowed these also to produce an F_3 crop by self-fertilization. In this case, 193 of the plants produced round seeds only. The other 372 plants produced both round and wrinkled seeds and in a 3 to 1 ratio (Fig. 17.3).

What is the significance of these facts? Clearly, when round-seeded peas are cross-bred with wrinkle-seeded peas, the round-seeded ones pass some controlling factor on to the offspring (F_1). Further, it does not matter whether the round-seeded factor is supplied by the male gamete or by the egg. The results are the same in either case.

The reappearance of wrinkled peas in the F_2 generation can only mean that at least some of the F_1 plants were also carrying a factor for the wrinkle-seeded condition. Its presence was, however, obscured in the F_1 generation. Traits which are transmitted unchanged to the F_1 generation (e.g. round seeds) Mendel called **dominant.** Those which are hidden in the F_1, but reappear in the F_2 (e.g. wrinkled seeds), he called **recessive.**

Dominant vs. recessive traits

17.4 MENDEL'S HYPOTHESIS

In an attempt to explain these facts, Mendel made a series of assumptions. These assumptions we call a hypothesis. They were not observations; they were not facts. They were simply statements which, if true, would provide an explanation for the observed results. They were:

1. In each organism there is a pair of factors which control the appearance of a given characteristic. (Today we call these factors **genes.**)
2. The organism gets these factors from its parents, one from each.
3. Each of these factors is transmitted as a discrete, unchanging unit. (The wrinkled seeds in the F_2 generation were no less wrinkled than those in the P_1 generation, although the factors controlling this trait had passed through the round-seeded F_1 generation.)

Mendel's first law

4. When the reproductive cells (sperm or eggs) are prepared, *the factors separate* and are distributed as units to each gamete. This is often called *Mendel's first law,* the *law of segregation.*

Alleles are alternative forms of a gene

5. If an organism has two unlike factors for a given characteristic, one may be expressed to the total exclusion of the other. Today, we use the term **allele** to describe *alternative forms of a gene* controlling a given

characteristic. Thus, in the case discussed, there are two alleles (round-seeded and wrinkle-seeded) of the gene which controls seed shape.

How well does this hypothesis explain the observed facts? According to Mendel's scheme, the pure-breeding round-seeded plants of the P_1 generation contained two identical genes for round seeds. We can designate these as *RR*. The pure-breeding wrinkle-seeded plants contained two of the wrinkle-seeded genes, *rr*. Today we say that each of the P_1 plants is **homozygous** for its respective trait. In the formation of gametes, the genes separate. Since the genes are alike in each plant, however, all the gametes of one plant are the same. Any sperm nucleus or egg from the round-seeded plant will carry the allele *R*. Likewise, any gamete from the wrinkle-seeded plant will carry the allele *r*. The only possible zygotes that can be formed when these two varieties are crossbred would contain both, *Rr*. All the cells of the F_1 plant thus carry one of each of the two alleles. Today we would say that each of the F_1 plants is **heterozygous.** All the F_1 seeds are round according to Mendel because in the heterozygous state, the allele *R* is expressed to the total exclusion of the allele *r*. In other words, *R* is dominant over *r*. We can describe this cross by means of a so-called Punnett's square (Fig. 17.4).

Homozygosity

Heterozygosity

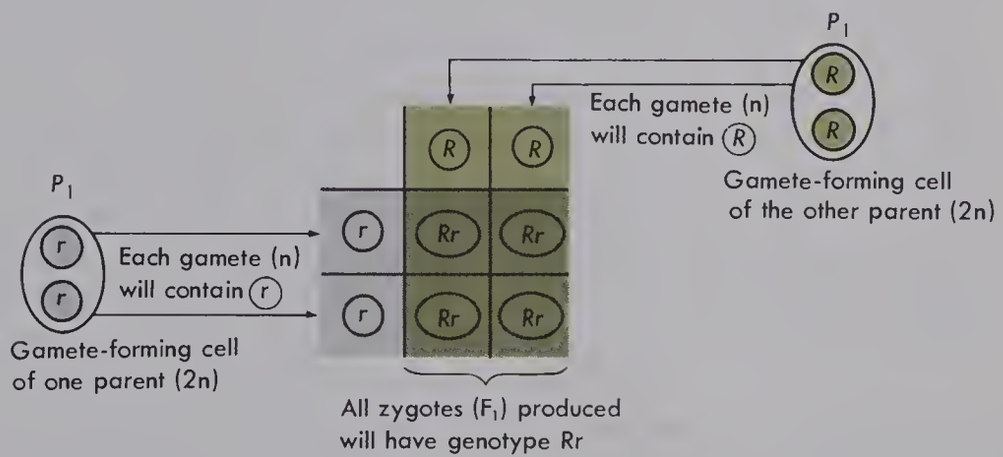


Fig. 17.4 How Mendel's hypothesis explains the results of mating a purebreeding wrinkle-seeded pea plant with a purebreeding round-seeded one.

When the F_1 plants form gametes, the alleles again separate, only one allele being transmitted to a given gamete. This means that half of the total number of gametes formed will contain the allele *R;* half will contain *r*. When these gametes are allowed to unite at random, roughly one-half of the zygotes will be heterozygous, one-quarter homozygous for *R,* and one-quarter homozygous for *r* (Fig. 17.5). Three different combinations are thus probable (*RR, Rr, rr*), and the expected ratio would be 1:2:1. Because of the dominance of *R* over *r,* however, there is no way to distinguish visibly between seeds containing *RR* and those containing *Rr.* Both would have round coats. Today we say that they have the same **phenotype,** that is, *appearance* with respect to some trait. However, they have different **genotypes,** *actual gene content,* for the trait. This explains the interesting results Mendel achieved when he grew his F_3 generation. All the wrinkled

Phenotype vs. genotype

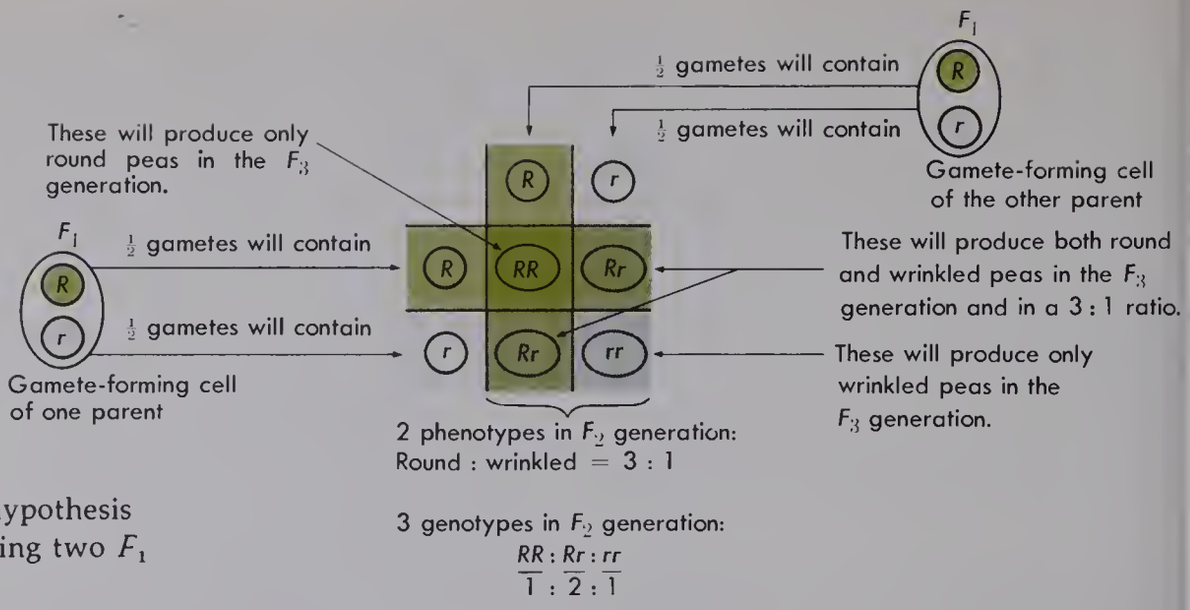


Fig. 17.5 How Mendel's hypothesis explains the results of mating two F_1 plants.

Actual seed production by each of ten of Mendel's F_1 plants

	Round	Wrinkled
1.	45	12
2.	27	8
3.	24	7
4.	19	16
5.	32	11
6.	26	6
7.	88	24
8.	22	10
9.	28	6
10.	25	7
Total	336	107

Fig. 17.6 Actual seed production by each of ten of Mendel's F_1 plants. Note that individual plants deviated widely from the expected 3:1 ratio but that the group as a whole approached it quite closely.

seeds bred true. One-third (193) of the round seeds also bred true, indicating that they were homozygous for *RR*. However, two-thirds (372) of the round seeds produced both round and wrinkled seeds and in a 3:1 ratio just as in the F_2 generation. Thus, they must have been heterozygous.

It is important to note that these ratios are only approximate. Far more pollen is produced than is ever used in fertilization. Many eggs may never be fertilized. One would no more expect that four F_1 fertilizations would always produce 1*RR*, 2*Rr*, and 1*rr* than one would expect a coin flipped four times to always come up heads twice and tails twice. But as the size of the sample gets increasingly large, chance deviations become minimized and the ratios approach the theoretical prediction more and more closely. Figure 17.6 shows the actual count of round and wrinkled seeds produced by ten of Mendel's 253 F_1 plants. In many cases, the ratio is far from the expected 3:1. By the time the seeds of all the plants have been counted, however, the ratio (3.14:1) is very close to that predicted.

17.5 HOW HYPOTHESES ARE JUDGED

Mendel's hypothesis provides a reasonable explanation for the results of his breeding experiments. While it is not the only scheme which can be devised to explain these results, it is the simplest scheme that anyone has yet thought up. We cannot ask if Mendel's hypothesis is true. All we can say is that it provides an adequate explanation of all the facts observed and that it is the simplest explanation which does this. "Reasonable," "adequate," "simplest" are all standards which are extraordinarily difficult to measure. Hypotheses, especially when newly created, must frequently be judged by just such subjective criteria as these, however. In the popular view, scientists lead working lives of complete objectivity. When dealing with facts, this is generally true. When dealing with hypotheses, however, they are also guided by subjective, aesthetic, even emotional consider-

ations. The reasoning proceeded in Mendel's case from the concrete details of numbers of different kinds of seeds to the generalizations that explain the facts, that "make sense" out of them. We call this kind of reasoning **inductive.** No simple rules of logic lead in this direction; one needs intuition or insight. The ability to make these inductive generalizations requires more art than science, but science would not exist without them.

Inductive vs. deductive reasoning

There is one further criterion that any good hypothesis should meet. It should be able to predict new facts. This prediction of new facts involves reasoning which is called **deductive.** If the generalizations are valid, then certain specific consequences can be deduced from them.

17.6 THE TESTCROSS: A TEST OF MENDEL'S HYPOTHESIS

Mendel appreciated fully the importance of this step. To test his hypothesis, he tried to predict the outcome of a breeding experiment he had never carried out. He crossed his heterozygous round peas (*Rr*) with homozygous wrinkled ones (*rr*). He reasoned that the homozygous recessive parent could only produce gametes with *r*. The heterozygous parent should produce equal numbers of *R* and *r* gametes. Mendel predicted that one-half of the seeds produced from this cross would be round (*Rr*) and one-half would be wrinkled (*rr*) (Fig. 17.7). This kind of mating, using as one parent a known homozygous recessive, is called a testcross. It "tests" the genotype in those cases where two different genotypes (like *RR* and *Rr*) produce the same phenotype. Note that to the casual observer in the monastery garden, the cross appeared no different from the P_1 cross described earlier. Round-seeded peas were being crossed with wrinkle-seeded peas. But Mendel, believing that the round-seeded pea plants in this cross were actually heterozygous, predicted that both round and wrinkled seeds would be produced and in a 50:50 ratio. He performed the matings and harvested 106 round peas and 101 wrinkled peas from his plants.

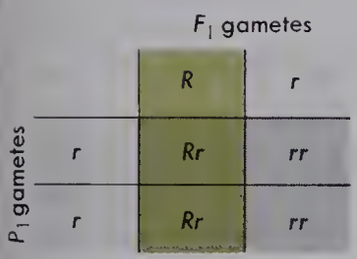


Fig. 17.7 Mendel's testcross. Although it superficially resembled a P_1 mating, Mendel's prediction that two phenotypes would be produced and in equal numbers was verified.

Mendel's hypothesis had explained all the known facts. It also led to the prediction of other facts as yet undiscovered. When these predicted facts were discovered, his hypothesis was greatly strengthened. A hypothesis that explains all the known facts in the situation and successfully predicts new ones is soon referred to as a **theory.** If a theory continues to serve its explanatory and predictive functions, it may eventually come to be called a **law.** Two of Mendel's assumptions (one of which we have already discussed) are now referred to as Mendel's laws.

law
↑
theory
↑
hypothesis

17.7 DIHYBRIDS—THE LAW OF INDEPENDENT ASSORTMENT

During the same period of time that Mendel was investigating inheritance in round- and wrinkle-seeded peas, he ran similar experiments with pea varieties differing in six other clear-cut ways. The results from all these other experiments also supported his hypothesis. Next, he crossed pea plants differing in two characteristics. A pure-breeding, round-seeded, yellow-cotyledon pea variety was cross-pollinated with a pure-breeding,

wrinkle-seeded, green-cotyledon variety. All the seeds produced from the mating were round and had yellow cotyledons. This bore out Mendel's previous finding that the allele for yellow cotyledons, like the allele for round seed shape, was dominant. (This F_1 generation is said to be *dihybrid* because it is produced by crossing parents differing in two traits.) Then Mendel planted these seeds and allowed the resulting flowers to self-pollinate. Either of two possibilities might be expected. The alleles for round shape and yellow cotyledons, which had been inherited from one parent, might be inseparable and thus passed on as a single unit to the F_2 generation. If the same were also true for the wrinkled-green alleles, one would expect that three-fourths of the F_2 generation would be round-yellow and one-fourth wrinkled-green (Fig. 17.8). If, on the other hand, the genes for seed shape and cotyledon color were distributed to the gametes independently of each other, then one would expect in the F_2 generation to find some peas that were round with green cotyledons and some that were wrinkled with yellow cotyledons as well as those resembling the P_1 types. According to this latter assumption, then, four phenotypes would be produced in the ratio 9:3:3:1 (Fig. 17.8).

The genes that Mendel studied were inherited independently of one another

Mendel performed this cross and harvested 315 round-yellow peas, 101 wrinkled-yellow peas, 108 round-green peas and 32 wrinkled-green peas. It is characteristic of Mendel's careful work that he then proceeded to plant all of these peas and verify the presence of four separate genotypes among the round-yellow peas and two separate genotypes among each of the peas with the new combination of characteristics. Only the 32 wrinkled-green peas were of a single genotype. These results led Mendel to frame his last hypothesis (Mendel's second law): the distribution of one pair of factors is independent of the distribution of the other pair. This has come to be known as his *law of independent assortment.*

17.8 MENDEL'S THEORY: THE SEQUEL

The experiments described in this chapter were carried on from 1858 to 1866. In 1866 Mendel published the results as well as his analysis of them. Little attention was paid to them by other biologists. No one tried to repeat any of the experiments or carry them on with different traits or different organisms. Mendel himself soon gave up his experiments and became increasingly involved with the administration of his monastery.

Mendel's laws were independently rediscovered in 1900

Mendel died in 1884. In 1900, thirty-four years after he had published his work and sixteen years after his death, Mendel's work was brought to light once more. Three men, working entirely independently of one another, discovered the same principles we have been describing. Only after their work was done did they discover that they had been anticipated by an obscure monk a third of a century before.

Several reasons have been proposed for the failure of Mendel's work to gain acceptance. Whatever the reasons, it did not. It is indeed ironical that the present development of genetics dates only from the year 1900, not 1866. Mendel's brilliant work failed to become part of the scientific

	RY	ry
RY	RRYY round -yellow	RrYy round -yellow
ry	RrYy round -yellow	rryy wrinkled -green

or

	RY	Ry	rY	ry
RY	RRYY	RRYy	RrYY	RrYy
Ry	RRYy	RRyy	RrYy	Rryy
rY	RrYY	RrYy	rrYY	rrYy
ry	RrYy	Rryy	rrYy	rryy

Round			Wrinkled	
yellow		green	yellow	green
9	:	3	: 3	: 1

Fig. 17.8 Alternative predictions of the results of mating two dihybrids. In this case, the second prediction (right) turned out to be correct.

world of his time. When scientists were ready to pursue his findings further, they had already rediscovered them on their own.

What is the status today of Mendel's *laws?* Although important exceptions to them have been discovered in the years since 1900, they still form the foundation upon which the science of genetics rests.

17.9 CONTINUOUS VARIATION: THE MULTIPLE-FACTOR HYPOTHESIS

Quantitative vs. qualitative variation

One of these exceptions arose from a study of the inheritance of traits which vary quantitatively among different individuals. Mendel had restricted his studies to traits which varied in a clear-cut, easily distinguishable, *qualitative* way. But men are not *either* tall *or* short; nor are they *either* heavy *or* light. Many traits differ in a continuous, quantitative way throughout a population (Fig. 17.9). Some of the variation can be explained by differences in diet and perhaps other factors in the environment. Environment is not, however, sufficient to explain the full range of heights or weights. No theory of inheritance could be regarded as completely successful until this kind of variation could be accounted for.

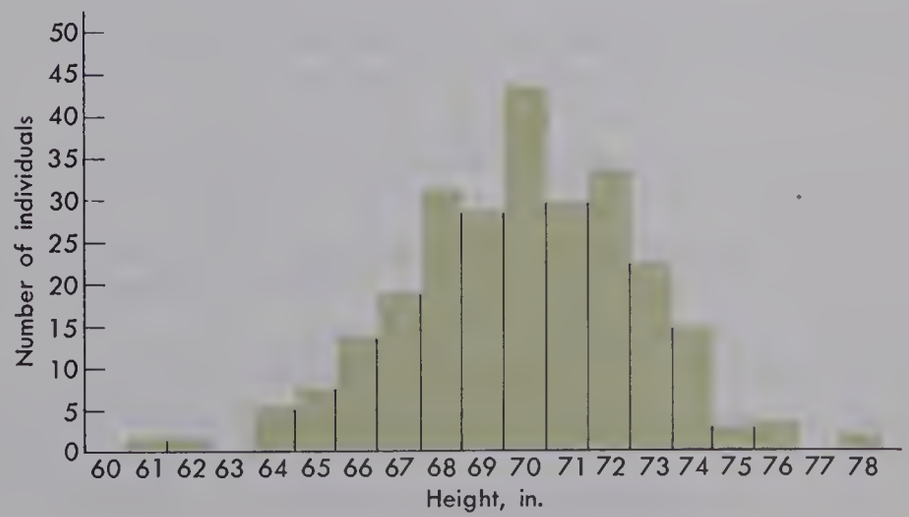


Fig. 17.9 Histogram showing distribution of heights among a group of male secondary-school seniors.

By 1908 the information was available which provided a solution to the problem. The Swedish geneticist, Nilsson-Ehle, studied the inheritance of kernel color in wheat. Using Mendel's methods, he mated pure-breeding red-kernel strains with pure-breeding white-kernel strains. The offspring were all red, but the intensity of the color was much less than in the red parent. It seemed as though the effect of the red allele in the F_1 generation was being modified by the presence of the white allele. This phenomenon, which had not occurred in Mendel's studies, is called **incomplete dominance.**

Incomplete dominance

Nilsson–Ehle's results

Furthermore, when Nilsson-Ehle mated two F_1 plants, he produced an F_2 generation in which red-kerneled plants outnumbered white-kerneled plants 15:1. Close examination revealed that the red kernels were not all alike. They could quite easily be sorted into four different categories. One-fifteenth of them were deep red, like the P_1 type. Four-fifteenths were medium dark red, six-fifteenths were medium red (like the F_1 generation) and four-fifteenths were light red. These results could be explained by assuming that kernel color in wheat is controlled by not one, but two pairs of genes, the effects of which add up *without* distinct dominance (Fig. 17.10). Four genes for red produce a deep red kernel. Four genes for white produce a white kernel. Just one red gene out of the four produces a light red kernel. Any two out of the four produce a medium red kernel. Any three out of the four produce a medium dark red kernel. If one plots the numbers of the different colored offspring in the F_2 generation against color intensity, a graph similar to that in Fig. 17.10 results.

In other wheat varieties, Nilsson-Ehle found F_2 generations with a ratio of red kernels to white of 63:1. These could be explained by assuming that three pairs of alleles were involved. In these cases, six different shades of red could be detected. Can you work out the genetics of this cross for yourself? The color differences were very slight. Environmental influences also caused alterations in intensity so that in practice the collection of kernels displayed a continuous range of hues all the way from deep red to white.

The occurrence of continuous variation of a trait in a population can thus be explained by assuming it is controlled by several pairs of genes, the effects of which are added together. This hypothesis is called the Multiple Factor Hypothesis. It suggests that (1) when two extreme types are mated (e.g. *AABB* and *aabb*), the offspring are intermediate in type; (2) when two intermediate types are mated, most of their offspring are also intermediate, but some extreme types will be produced; and (3) the results of random matings in a large population will be a wide range of types with the greatest number in the middle range and the fewest at the extremes. These three effects are actually observed in most cases of quantitative variation in living things.

Predictions of the multiple-factor hypothesis

When Mendel carried out his experiments on heredity, the nature of the nucleus, the chromosomes, and meiosis was not understood. In formulating the idea of genes, Mendel made no attempt to describe them as physical structures or to say precisely where in the gametes they were

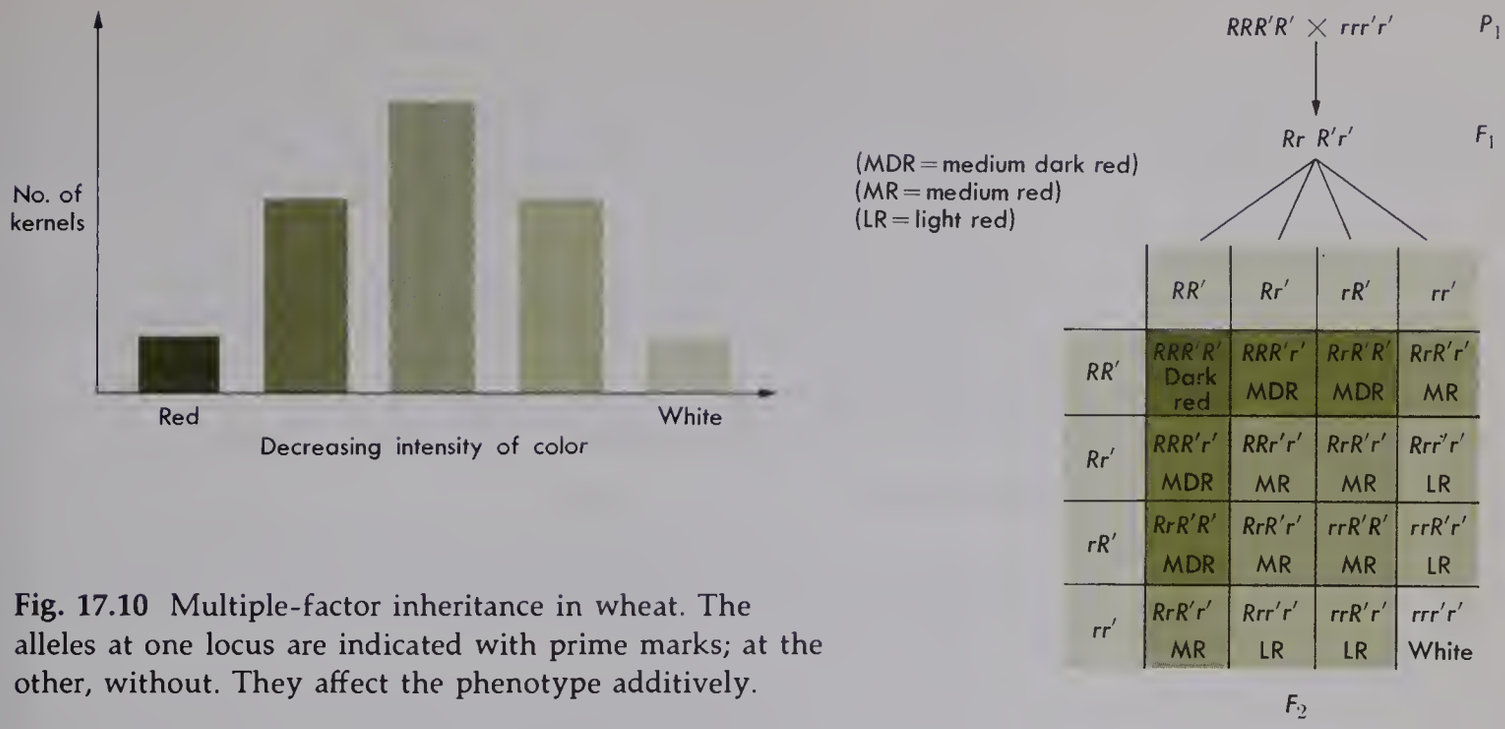


Fig. 17.10 Multiple-factor inheritance in wheat. The alleles at one locus are indicated with prime marks; at the other, without. They affect the phenotype additively.

located. For Mendel, the genes were simply hypothetical entities which explained observed patterns of inheritance. With the rediscovery of his work, biologists immediately attempted to relate the behavior of his genes to visible features in the cell. Their efforts will be the topic of the next chapter.

EXERCISES AND PROBLEMS

1. What terms did Weismann use to distinguish the body cells of an animal from its reproductive cells?
2. Do these terms apply equally well to bacteria? To plants?
3. Give the gene content of the different kinds of eggs produced by a woman whose genotype is JjKkLl.
4. Is it correct to say that crossing two heterozygous red-flowered peas will produce eight seeds, two of which will be homozygous for the trait? Explain.
5. The gene for tallness in peas is dominant over the gene for dwarfism. A cross between a tall pea and a dwarf pea produced 86 tall plants and 81 dwarf plants. What was the probable genotype of the tall plant? What is this kind of cross called?
6. Why was Mendel's choice of the pea plant as an experimental organism such a good one?
7. Distinguish between fact and a hypothesis. Are the two always easily distinguishable?
8. In *Drosophila* sepia eye is recessive to red eye, and curved wing is recessive to straight wing. If a pure-breeding sepia-eyed, straight-winged fly is mated with a pure-breeding red-eyed, curve-winged fly, what phenotypes will appear in the F_1 generation? If two F_1 flies are allowed to mate, what phenotypes will occur in the F_2 generation and in what ratio?

9. How many different genotypes will occur in the F_2 generation described in Question 8?
10. When plant A (pure-breeding tall) is crossed with plant B (pure-breeding short), the offspring are all intermediate in height. When two of the offspring are crossed, 1/16 of the next generation is as tall as the tall grandparent. How do you account for this?
11. While continuous variation can be accounted for by multiple factor inheritance, cases involving three or more pairs of genes have been difficult to demonstrate conclusively. Why?
12. Define a gene.

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18 THE CHROMOSOME THEORY OF HEREDITY

Parallel Behavior of Genes and Chromosomes

Sex Determination

Sex Linkage

Chromosome Abnormalities

Linkage

Chromosome Maps

The Evidence of Creighton and McClintock

Assigning Linkage Groups to Chromosomes

The 35 years during which Mendel's discoveries lay unnoticed saw many important discoveries in cytology—the study of cells. The importance of the nucleus in controlling the traits of the organism was revealed by Boveri's work with sea-urchin eggs, discussed in Chapter 5. The behavior of the chromosomes in mitosis and meiosis, discussed in Chapter 16, was also discovered.

18.1 PARALLEL BEHAVIOR OF GENES AND CHROMOSOMES

Were geneticists and cytologists examining two aspects of the same problem?

Shortly after the rediscovery of Mendel's "laws," several workers, among them Sutton and Boveri himself, realized that certain of Mendel's rules of gene behavior could be explained if the genes were located on or in the chromosomes. Mendel had said that genes occur in pairs, one member of which is received from each parent. Chromosomes also exist in pairs, one member of which is received from each parent. Mendel had said that when the reproductory cells are produced, the paired factors (genes) separate and are distributed as units to each gamete (*law of segregation*). In meiosis, the homologous pairs of chromosomes separate and only one member of each pair goes to a given sex cell. Mendel had stated further that in a dihybrid cross, the distribution of one pair of factors is independent of the distribution of the other pair *(law of independent assortment)*. In meiosis, the distribution of maternal and paternal chromosomes is entirely at random. If we assume that the genes for one trait, say seed shape, are located on one pair of chromosomes and the genes for the other trait (cotyledon color) on another pair, then the independent assortment of the chromosome pairs in meiosis will also result in the independent assortment of the two traits (Fig. 18.1). Because there are so many more traits in an organism than there are

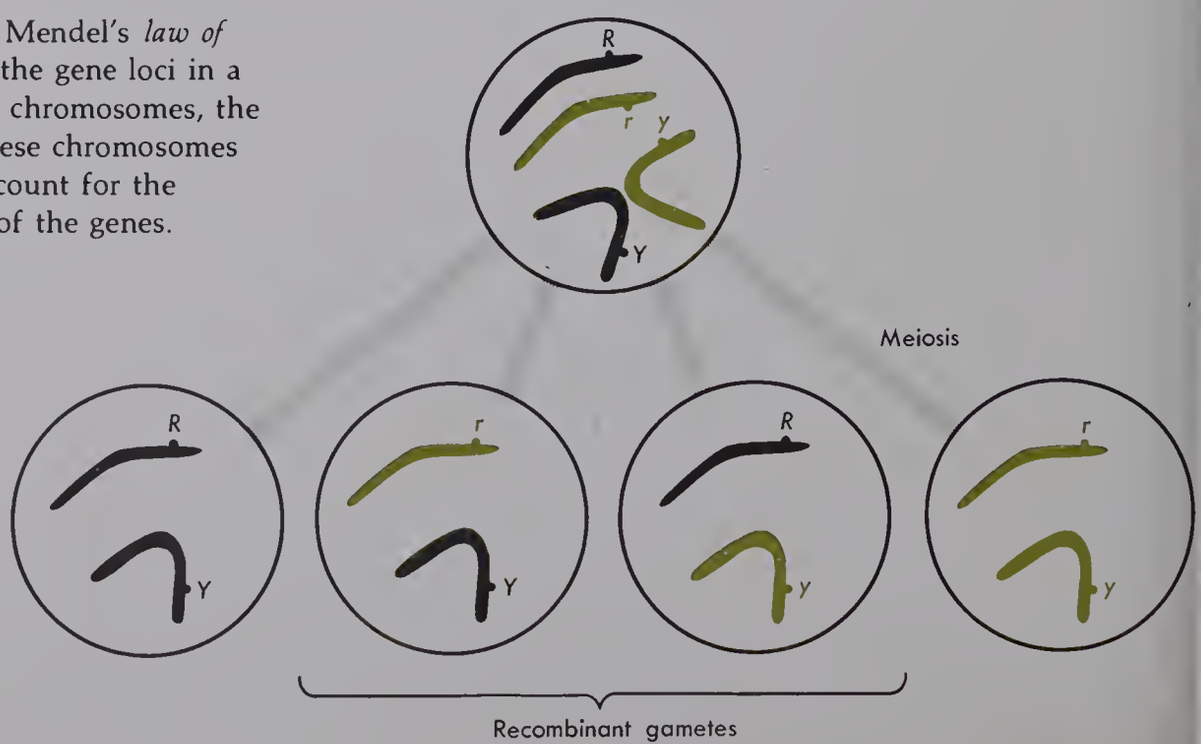


Fig. 18.1 Mechanism of Mendel's *law of independent assortment.* If the gene loci in a dihybrid are on different chromosomes, the random assortment of these chromosomes during meiosis would account for the independent assortment of the genes.

chromosomes, the chromosomes cannot be identical to the genes. Instead each chromosome must contain many genes. If we mated dihybrids in which both traits were controlled by genes in the same chromosome, would you expect Mendel's *law of independent assortment* to still hold? Later in the chapter we shall see that many dihybrids are of this sort and this law does not hold for such cases. Instead, these genes are said to show "linkage."

The chromosome theory

The fact that the behavior of the hypothetical units of heredity (genes) is parallel to the behavior of the chromosomes that can actually be seen under the microscope suggested to Sutton that the genes are located in or on the chromosomes. He reasoned that the two alleles controlling a given trait are located at corresponding sites (**loci**—singular, **locus**) on each of the two homologous chromosomes. While the parallel behavior of genes and chromosomes suggested that they were associated in this way, it did not prove that this was so.

How to verify the chromosome theory?

For more direct proof it was necessary to be able to associate the presence or absence of a given trait or traits in an organism with the presence or absence of a given chromosome in the body cells of this organism. But the two alleles controlling the expression of a given trait are, according to the chromosome theory, located at corresponding loci on each of two homologous chromosomes. Homologous chromosomes may be visually distinguishable from other chromosomes in the cell but are not distinguishable from each other. So it is not possible to tell by visual inspection of a member of the pair whether it carries a given allele or not.

TESTS OF THE CHROMOSOME THEORY

18.2 SEX DETERMINATION

Advantages of Drosophila *as an experimental animal*

The first solution to this dilemma came as a result of studies on the "fruit" fly *Drosophila melanogaster* (Fig 18.2). Thomas Hunt Morgan pioneered in the use of this tiny organism as a subject of research in genetics. It was an admirable choice for several reasons. First, the flies are small and thus sizable populations of them can be reared easily in the laboratory. Second, the life cycle is completed quite rapidly. A new generation of adult flies can be produced every two weeks. Third, the flies are remarkably fecund; that is, a female may lay hundreds of fertilized eggs during her brief life span. The large populations thus produced make statistical analyses easy and reliable. Still a fourth virtue was discovered: the presence of giant chromosomes in the salivary glands of the larvae. These chromosomes show far more structural detail than do normal body chromosomes (Fig. 18.3). Although the pattern of bands may not represent genes themselves, eventually it became possible to associate specific genes with specific bands in the chromosomes.

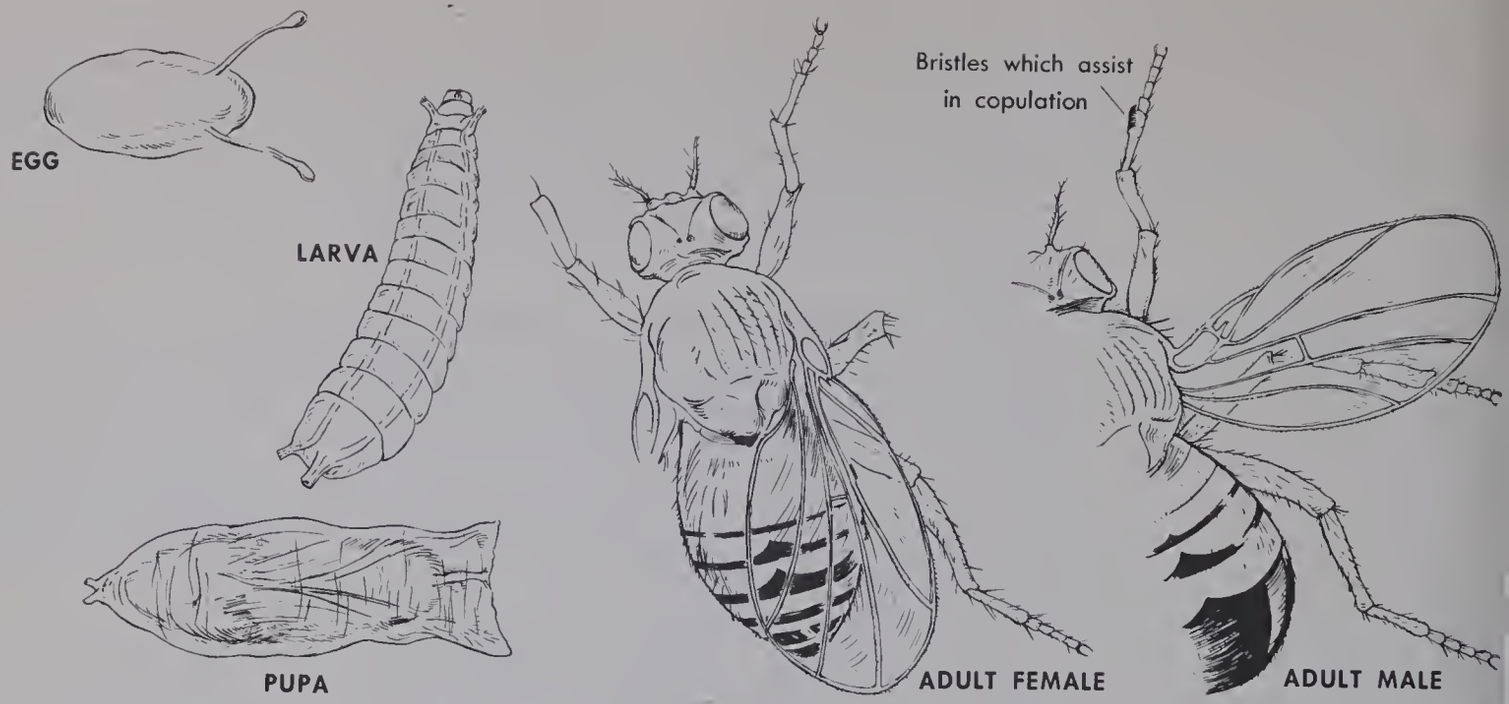


Fig. 18.2 Stages in the development of the fruit fly *Drosophila melanogaster.*

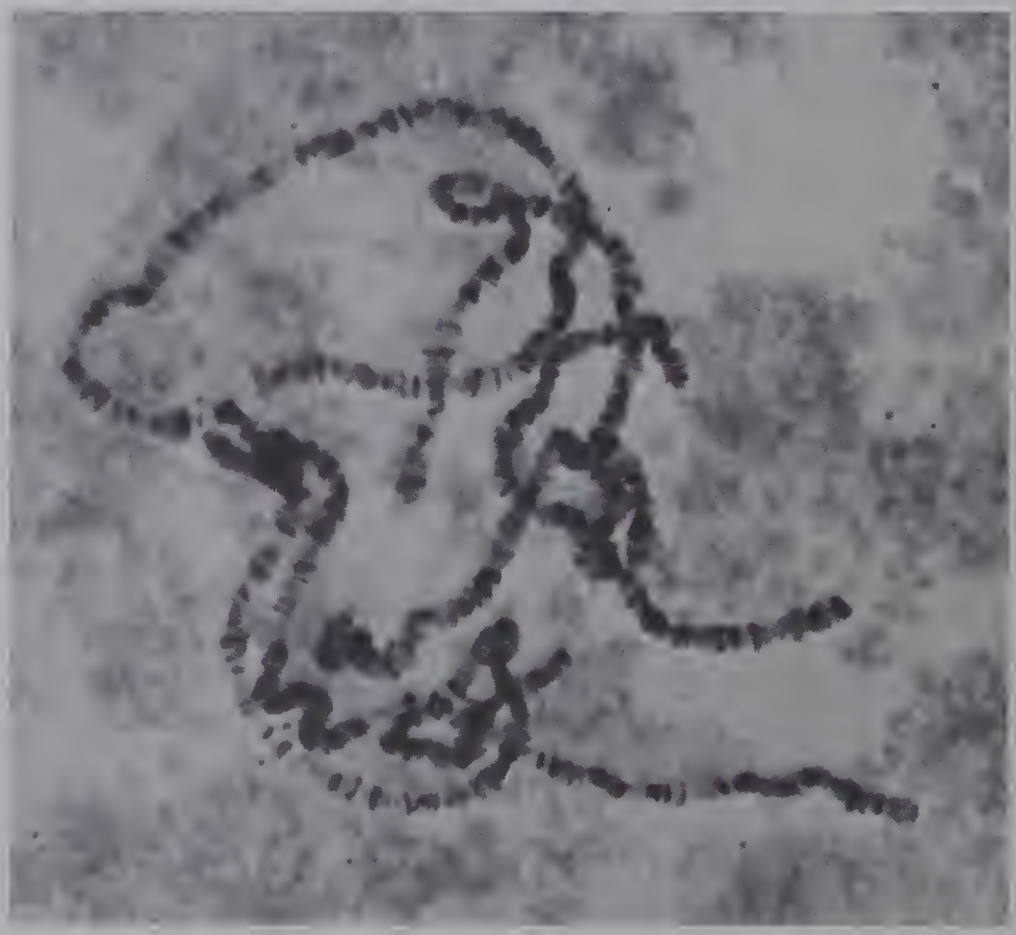


Fig. 18.3 Giant chromosomes from the salivary glands of the fruit fly *Drosophila melanogaster.* Such chromosomes are found in other large, active cells as well. (Courtesy of the General Biological Supply House, Inc.)

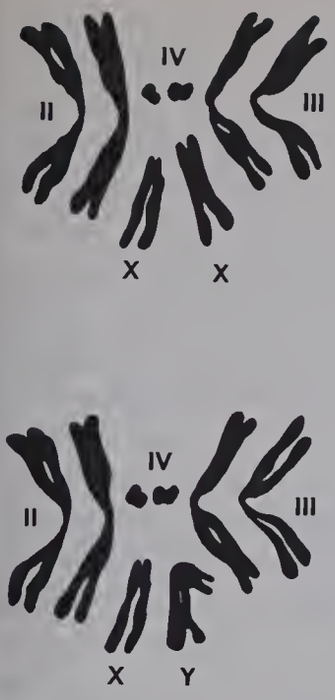


Fig. 18.4 Chromosomes of *Drosophila melanogaster.* Both sexes have three homologous pairs of autosomes. In addition the females have two *X* chromosomes (top), the males have an *X* and a *Y* chromosome (bottom). These are the sex chromosomes.

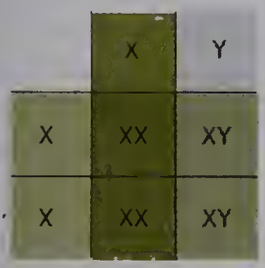


Fig. 18.5 Sex determination. A fruit fly (or human) with 2 *X* chromosomes is female; males have 1 *X* and 1 *Y* chromosome.

Although a female fruit fly has four pairs of homologous chromosomes (Fig. 18.4), the male fly has only three pairs of homologous chromosomes. The remaining two chromosomes are not homologous. One of the fourth pair of chromosomes is identical in appearance to the chromosomes of the fourth pair in the females. It is called the *X* chromosome. The other chromosome in the male is quite different in appearance. It is called the *Y* chromosome (Fig. 18.4). Collectively, these *X* and *Y* chromosomes are called **sex chromosomes** because their presence is always correlated with the sex of the fly. The other chromosomes are called **autosomes.**

As a result of the separation of homologous pairs in meiosis, fruit-fly *eggs* contain one of each of the autosomes plus one *X* chromosome. The sperm cells produced by male fruit flies contain three autosomes and either an *X* or a *Y* chromosome. We can depict the results of random union of these sperm and eggs by setting up a Punnett's square (Fig. 18.5). (Note that the symbols now refer to whole chromosomes, not genes.) One can quickly see that the offspring should be about equally divided between males and females, and this indeed is the case.

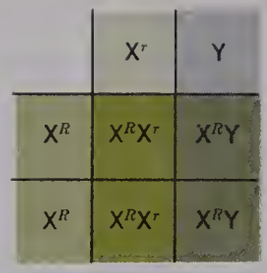
Glancing at Fig. 18.2, we can immediately see that the matter of sex differences is one of inherited traits. The pigmentation on the abdomen of the male, the penis, the bristles on the first tarsal segment of the foreleg are just a few of the visible traits that distinguish the male fruit fly from the female. The mere fact that the presence or absence of these traits is always related to the sex chromosomes present is evidence of the chromosome theory of heredity.

It is tempting to think of the *Y* chromosome as carrying the genes which determine maleness. In mammals, including man, this is the case. In many insects, including the fruit fly, however, the *Y* chromosome seems to be genetically inert. Only a few genes have been associated with it and these are not directly related to sex. Probably the autosomes carry the male determinants and it takes two *X* chromosomes to mask their effect and produce a female. The fact that the male grasshopper has no *Y* chromosome at all, just one *X* chromosome, while the female has two, is one bit of evidence to support this idea. In birds, the *XY* chromosome constitution is found in the females, the *XX* in males. Reptiles appear to be like birds in this respect. Among the amphibians and fishes, we find sometimes one system, sometimes the other.

Whatever the specific mechanism involved, sex in the animal kingdom seems to be directly related to the arrangement of specific chromosomes and thus serves to support the general theory that the chromosomes carry the determinants of the traits of an organism.

18.3 SEX LINKAGE

The discovery of the mechanism of sex determination in fruit flies quickly paved the way for another test of the chromosome theory, this one involving the inheritance of one specific trait. In one of Morgan's *Drosophila* cultures there appeared a male fly with white eyes instead of the brilliant



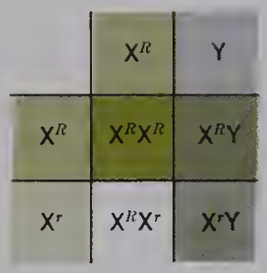


Fig. 18.6 Sex linkage. Any recessive allele (e.g., *r*-white eyes) on the *X* chromosome will be expressed in males because no corresponding gene loci are found on the *Y* chromosome.

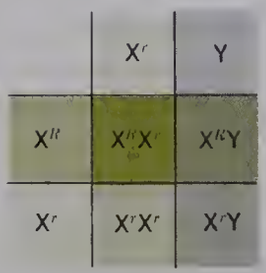


Fig. 18.7 Morgan's testcross. By mating a white-eyed male fruit fly with a "carrier" female, Morgan was able to produce white-eyed females.

red eyes characteristic of the species. When this fly was mated with normal red-eyed females, the offspring all had red eyes. This suggests that if the white-eyed trait is determined by a specific gene, it must be recessive. According to Mendel's *law of segregation,* all the red-eyed F_1 offspring must have been hybrid for the trait. (Why?) We can then predict that if two red-eyed F_1 flies were mated, white-eyed flies would appear in the F_2 generation in about 25% of the offspring. When Morgan made this cross, he found that this indeed occurred but all the white-eyed offspring were males. Not a single white-eyed female appeared. Morgan reasoned that this peculiar sex-related type of inheritance could be explained if one assumed that the alleles involved were located on the *X* chromosomes. The female flies, having two *X* chromosomes, would have to be homozygous for white eyes to show the trait. The males, on the other hand, having only one *X* chromosome, would show the trait for whichever allele they had on that chromosome. Even if it were the recessive allele *(r),* the *Y* chromosome contains no allele that would be dominant to it. Figure 18.6 shows diagrammatically Morgan's idea of what had occurred in his first two crosses. Note again that the symbols *X* and *Y* stand for the visible chromosomes and the superscripts *R* and *r* for the dominant and recessive alleles, respectively.

If this hypothesis is correct, it should be possible to produce white-eyed females by mating white-eyed males with F_1 females. Morgan made this cross and produced all four kinds of flies in approximately equal numbers (Fig. 18.7). Today we call this type of inheritance *sex-linked.* In humans, red-green color blindness, the blood disease hemophilia, and some 80 other known traits are produced by sex-linked genes. These traits are rare in females because they must inherit the recessive alleles from both their father and their mother in order to show the trait. This would be especially unlikely in the case of hemophilia because a man possessing the allele is a hemophiliac or "bleeder," and until recently his chances of ever becoming a father were quite dim. Figure 18.8 shows a pedigree of the descendants of Queen Victoria. Note that the allele for hemophilia, which has plagued the royal houses of Europe since her time, was nearly always passed on by the mothers who were heterozygous for the trait and thus showed no symptoms. We call them "carriers" because, while free from symptoms, they passed the recessive allele on to approximately one-half their sons, who became bleeders.

A good deal of disability and grief could be avoided if methods were found by which "carriers" of genetic defects could be detected. Knowledge of their genetic status would provide a basis on which to evaluate the risk of passing a defect on to their children. For many genetic defects (including hemophilia) the presence of one "good" gene permits normal functioning, i.e. the good gene appears to be fully dominant. However, with the development of delicate biochemical tests, it is now often possible to detect a reduced level of gene activity in carriers. In some cases, this is probably because one gene cannot do the job quite as well as two. In the case of

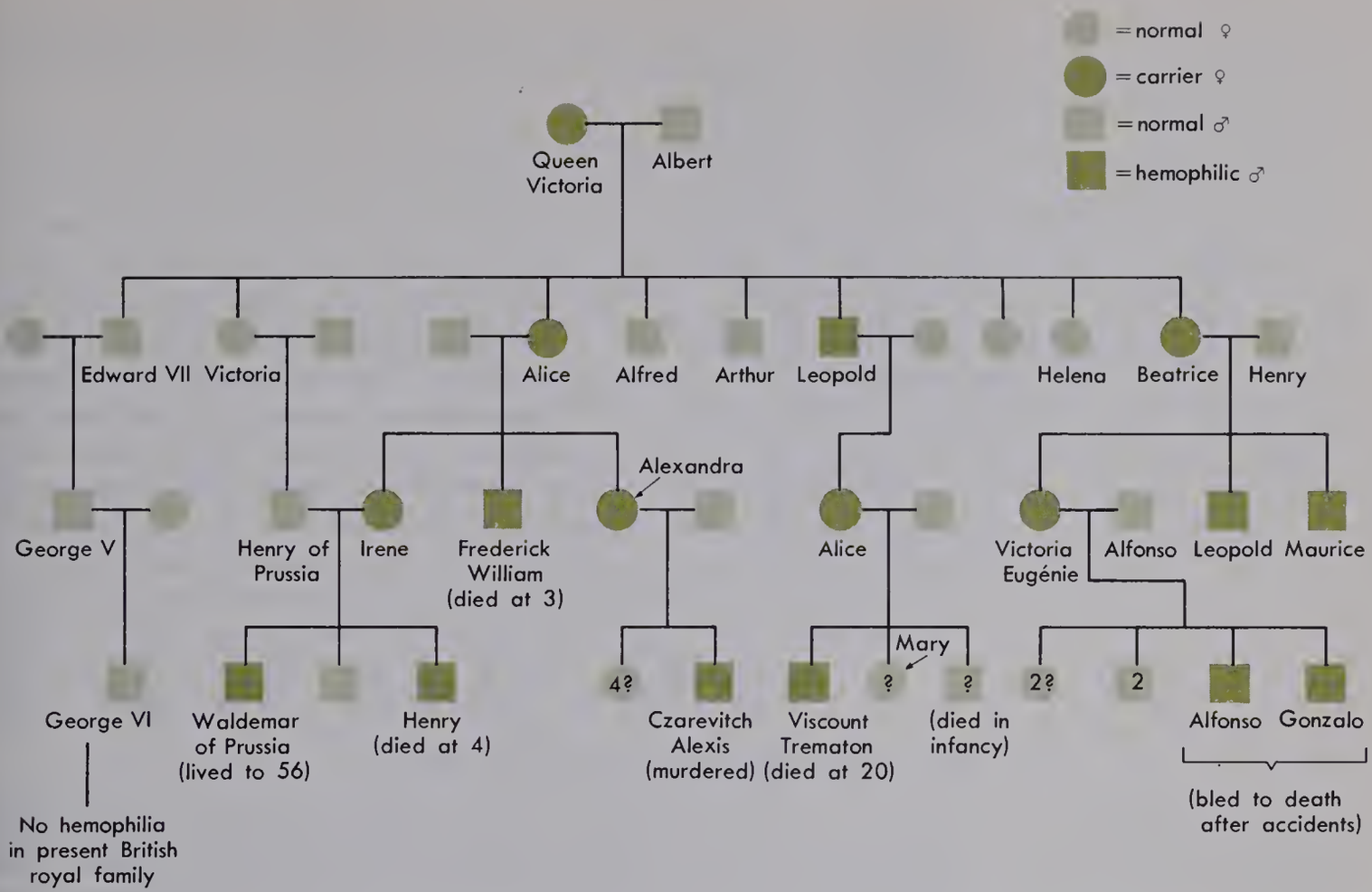
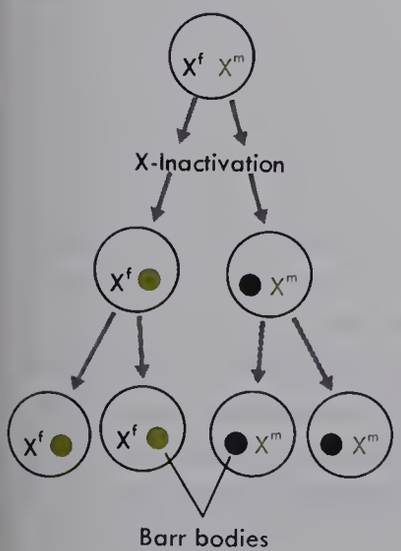


Fig. 18.8 Pedigree showing inheritance of hemophilia, a sex-linked trait, in the descendants of Queen Victoria. Many of the descendants in the third and fourth generations (3rd and 4th rows) have been omitted because the mutant gene was not transmitted to them.

sex-linked genes, however, another mechanism comes into play. This is the inactivation which occurs to *one* X chromosome in each female cell.

During interphase, the chromosomes are too tenuous to be stained and observed by microscopy. However, a dense, stainable structure is found to be present in virtually all the interphase nuclei of *female* mammals. It is not found in males. This structure (called a **Barr body** after its discoverer) turns out to be one of the X chromosomes. Its compact appearance is a reflection of its inactivity.



It now appears that early in embryonic development, one or the other of a female's X chromosomes becomes inactivated and converted into a Barr body. Which of the two X chromosomes becomes inactivated in a given cell is probably a matter of chance (except in marsupials like the kangaroo, where it is the X chromosome inherited from the father that is inactivated). However, after inactivation has taken place, all the descendants of that cell will have the same chromosome in the inactive state. We may again use the term *clone* to describe the descendants of such a cell.

Although all the cells in one individual probably have the same absolute gene content, *X* inactivation creates subpopulations (clones) with differing *effective* gene content.

Several sex-linked genetic defects are known that involve deficiencies in enzyme synthesis. When cells taken from women who are carriers for such traits are grown in tissue culture, we should expect to find some cells that show the defect and some that do not. In at least four such cases, it has been possible to demonstrate the existence of two such populations. Clones that fail to express enzyme activity are presumably descendants of a cell in which the normal gene, and the *X* chromosome of which it is a part, became inactivated. An organism whose cells vary in *effective* gene content and hence in the expression of a trait is said to be a genetic *mosaic.*

Genetic mosaics

The discovery and analysis of sex-linked traits, and of their peculiar mosaic expression in certain cases, serves to corroborate further the chromosome theory of inheritance. Each example described can now best be explained by assuming that the alleles involved are located on a specific chromosome.

18.4 CHROMOSOME ABNORMALITIES

The total chromosome complement of a cell is its karyotype

Karyotype analysis (Fig. 18.9) has revealed that in about 3% of human pregnancies, the cells of the child contain abnormal chromosomes. These abnormalities are often associated with severe deformities, although this is not always the case. The chromosomal abnormalities fall into two major groups. These are (1) alterations in chromosome number and (2) alterations in the structure of one or more chromosomes. In man, most of the disorders of chromosome *number* arise because of **nondisjunction.** You remember that in meiosis the homologues separate, one going to each pole of the cell. On rare occasions, a given pair of homologous chromosomes fails to separate (nondisjunction). As a result, one daughter cell gets both homologues and the other daughter cell gets neither.

Non-disjunction

If the sperm or egg cell that develops from these aberrant cells is used in fertilization, it will transmit its chromosome excess or deficiency to the zygote. Approximately 0.2% of newborn children suffer from Down's syndrome (also called mongolism), which is characterized by a number of defects including severe mental retardation and a high susceptibility to leukemia. Karyotype analysis of these unfortunate individuals reveals 47 chromosomes instead of the usual 46. The extra chromosome is a number 21 (Fig. 18.9). One way in which the zygote can receive three number 21 chromosomes is nondisjunction during egg formation, as a result of which an egg gets both of the mother's number 21 chromosomes. The chance of this occurrence seems to increase with age, older women being somewhat more likely to produce children with this defect than younger ones. The occurrence of children with three number 13 chromosomes or three number 18 chromosomes has also been detected. Each defect is accompanied by severe developmental abnormalities.

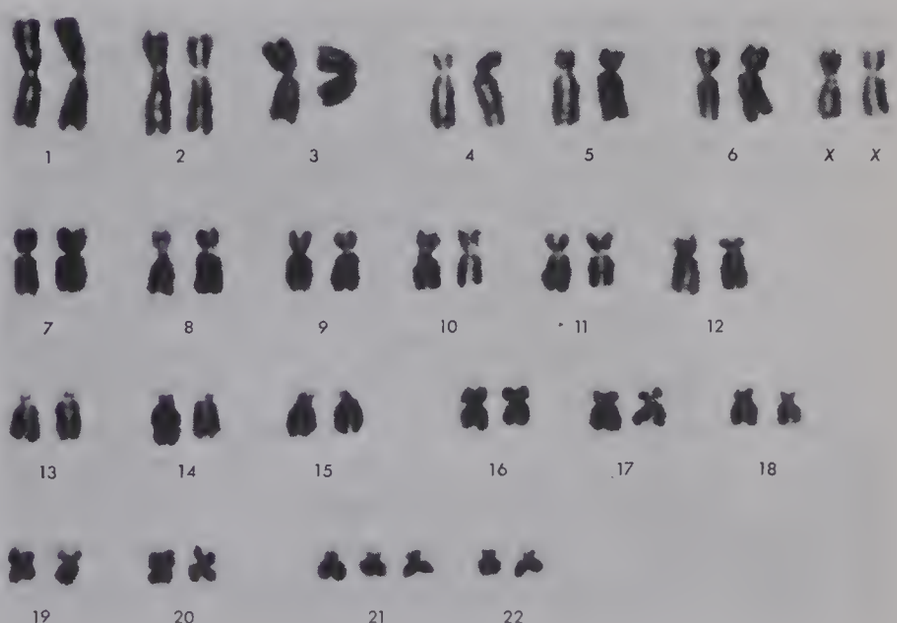
Down's syndrome



Fig. 18.9 (a) The 46 chromosomes of a human female. The two *X* chromosomes are marked. (Courtesy of Drs. T. T. Puck and J. H. Tjio.) Karyotypes can be conveniently analyzed by cutting the photograph into pieces containing single chromosomes and arranging these by homologous pairs (b and c, courtesy of Dr. James L. German, III).



(b) This is the karyotype of a normal male. The *X* and *Y* chromosomes are marked.



(c) This is the karyotype of a victim of Down's syndrome (mongolism). There are *three* number 21 chromosomes, making a total of 47. What was the sex of this individual?

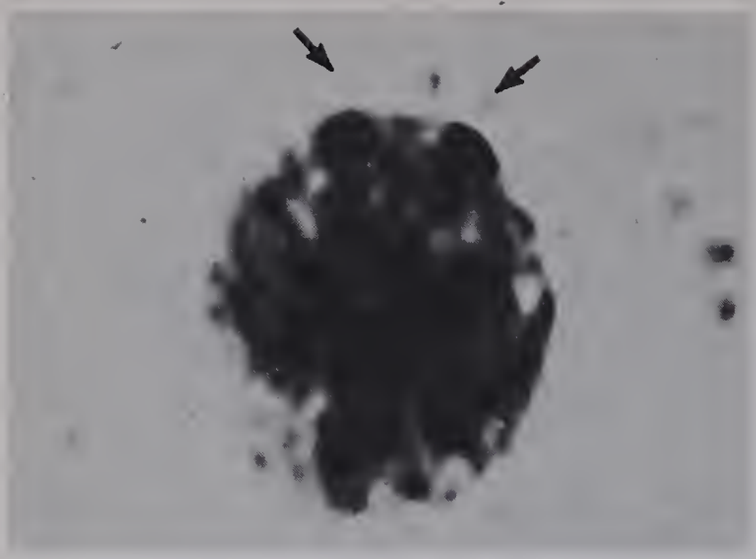


Fig. 18.10 Cheek cell from a woman with three *X* chromosomes instead of the usual two. Two Barr bodies can be seen (at top). (Courtesy of Dr. Kurt Hirschhorn.)

Abnormalities in the number of sex chromosomes

Abnormal numbers of sex chromosomes also occur at a low frequency. These chromosome abnormalities do not produce such gross deformities as do abnormal numbers of autosomes. Individuals with *XXY* and *XXXY* karyotypes develop as males (because of the presence of the *Y*) but are sterile. (The condition is known as Klinefelter's syndrome.) *XYY* males are also known and studies both in England and the United States indicate that this chromosome constitution is associated with tallness (6 feet or more) and, perhaps, a tendency to destructive or criminal behavior.

Supernumerary X chromosomes are converted into Barr bodies

Females with a single *X* are known. While somewhat abnormal, the fact that they are viable at all lends support to the idea discussed earlier in the chapter, that only one *X* chromosome is functional in female cells. This would also explain the relative benignity of such chromosome constitutions as *XXX*, *XXXX*, and even *XXXXX*, all of which have been found in females. Whereas normal women have one *X* chromosome inactivated and hence one Barr body, these women have all their extra *X* chromosomes inactivated as well. The number of Barr bodies in their cells is equal to the number of *X* chromosomes minus one (Fig. 18.10).

Polyploidy

Among plants and, more rarely, among animals we sometimes find another abnormality of chromosome number, called **polyploidy.** If after the duplication of the chromosomes in a diploid ($2n$) cell, the *cell* itself fails to divide in two, the nuclear membrane will reform around four sets of chromosomes instead of the usual two sets. Such a cell is tetraploid ($4n$) rather than diploid. A cell containing any multiple of the haploid number of chromosomes (above two) is said to be polyploid. Polyploidy is often found in natural plant populations. There is a species of grass in New Zealand that is 38-ploid ($38n$, with $n = 7$).

Induction of polyploidy

Polyploidy can also be induced by treating dividing cells with the substance colchicine. Colchicine disrupts microtubules and thus prevents formation of the spindle. As a result, the duplicated chromosomes fail to separate into the usual two groups at the poles. Onion cells exposed to colchicine for several days have been found with over a thousand chromosomes inside.

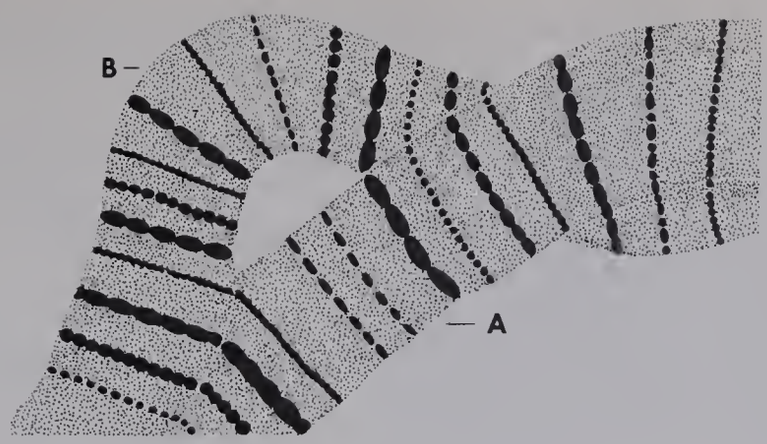
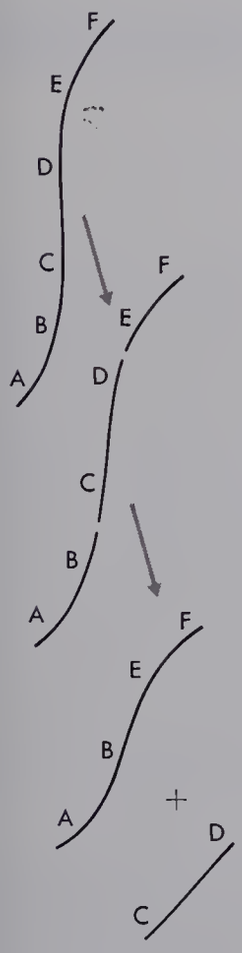


Fig. 18.11 Synapsis of a chromosome having (A) a deletion with (B) its normal homologue. The buckled portion in (B) corresponds to the deleted portion of (A).

Polyploidy in plants is often associated with larger size and greater vigor. For this reason, plant breeders have developed a number of polyploid varieties of commercially important species including corn, watermelons, marigolds, and snapdragons.

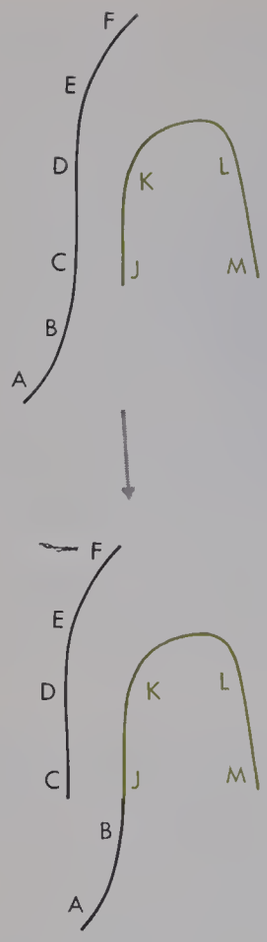
Structural alterations in chromosomes seem to occur when a break in a chromosome is followed by a loss or rearrangement of the broken parts. For example, after such a break, a segment of the chromosome may become lost, resulting in a **deletion.** When a chromosome carrying such a deletion undergoes synapsis with its homologue, only corresponding portions come together. The result is a loop in the normal chromosome (Fig. 18.11). An organism may reveal such a deletion by a change in its phenotype. For example, a recessive allele on the normal chromosome will be expressed if its dominant allele was located in the missing segment of the homologue with the deletion. One form of leukemia in humans is associated with the presence of a deletion on a number 22 chromosome in the white blood cells. To be able to associate a specific trait with a microscopically observable chromosome provides strong support for the chromosome theory.



Deletion

Chromosome breaks may also lead to inversions and translocations. In an **inversion,** a segment of a chromosome is turned around *(ABCDE⟶ ADCBE).* Inversions seldom have drastic effects on the phenotype of their owner because all the genes are still present. Difficulties arise, however, when a chromosome with an inversion attempts synapsis and crossing over with its normal homologue during meiosis. Sex cells with defective chromosomes will be produced, leading to partial sterility or serious defects in the offspring. Inversions are easy to detect in giant chromosomes, such as those in the salivary glands of the fruit fly, and were first seen in them. However, recent advances have now made it possible to identify inversions in human chromosomes as well.

Occasionally a segment of one chromosome breaks loose and becomes attached to a chromosome with which it is not homologous. This is called a **translocation.** Organisms with translocations also have fertility problems because half or more of the gametes formed by meiosis will have deletions and may not be able to create viable offspring. Translocations are



Translocation

now commonly found in human chromosomes, usually in people who have had repeated stillbirths or children with serious developmental defects.

A large number of agents have been identified that cause chromosome breaks. These include x-rays and other types of ionizing radiation. Radiation-induced breaks are quite random, that is, they may occur anywhere on a chromosome.

Many chemicals induce chromosome breakage. These include inhibitors of DNA synthesis and certain antibiotics. Most chemically induced breaks occur at specific parts of the chromosome, often near the centromere or other constricted parts of the chromosome. A great deal of publicity has surrounded the discovery that the psychotomimetic drug lysergic acid diethylamide (LSD) causes nonrandom chromosome breaks in laboratory animals and has been *associated with* such breaks in psychiatric patients receiving the drug. Whether pure LSD in doses apt to be ingested by casual users of the drug represents a hazard *to the chromosomes* is still a matter of controversy.

Chromosome breakage and rearrangements are also associated with virus infections. Measles, chicken pox, and viral meningitis infections have all been implicated.

18.5 LINKAGE

Earlier in the chapter, it was pointed out that the large number of genes and the relatively small number of chromosomes in an organism require that each pair of chromosomes possess many gene loci. In considering the inheritance of two different traits controlled by genes located on the same chromosome, it is obvious that Mendel's *law of independent assortment* can no longer hold. You will remember from the previous chapter that Mendel developed several strains of peas which were dihybrids; that is, they were heterozygous for two different traits, such as seed shape and cotyledon color. When a dihybrid plant *(Rr Yy)* was mated with the homozygous recessive P_1-type (a testcross), the resulting generation contained not only individuals of the P_1-types, round-yellow and wrinkled-green, but also individuals displaying new combinations of the traits, i.e. round-green and wrinkled-yellow. These latter individuals are called *recombinants.* The four kinds of offspring were produced in approximately equal numbers. Today we know that this independent assortment of genes occurred because the gene loci for the two traits were located on different pairs of chromosomes. Because of the random arrangement of maternal and paternal chromosomes on either side of the equatorial plate in first meiosis, random distribution of the genes resulted. This must have been true of all the cases that Mendel studied and as a result, he framed his second law, the *law of independent assortment:* the distribution of one pair of factors is independent of the distribution of the other pair.

Mendel's law of independent assortment holds only for genes on different pairs of chromosomes

Shortly after the rediscovery of Mendel's work, it became apparent that his second law does not account for inheritance in many dihybrids.

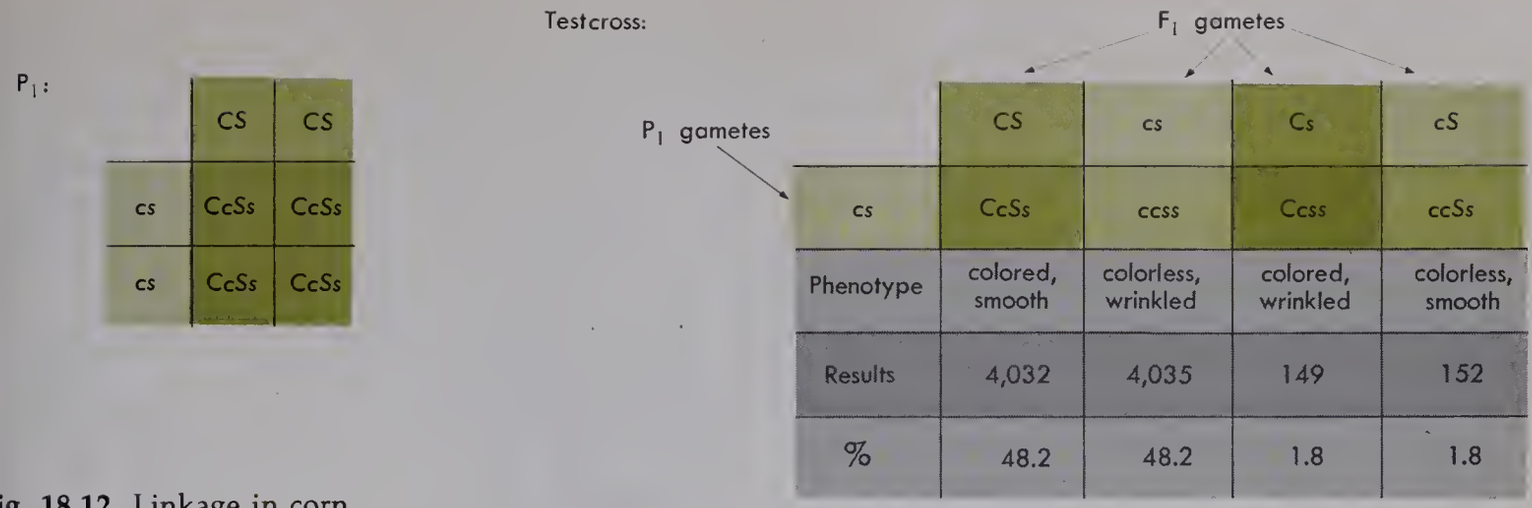


Fig. 18.12 Linkage in corn.

Demonstrating linkage

In many cases, two alleles inherited from one parent show a strong tendency to segregate together in the formation of gametes and the two alleles inherited from the other parent do the same. This phenomenon is called **linkage.** One of the clearest examples of linkage is found in corn. A strain of corn can be developed which produces kernels that are yellow in color and well filled with a food reserve tissue called endosperm. This strain breeds true, indicating that it is homozygous for these traits. Another strain can be developed which breeds true for colorless kernels that are quite wrinkled in appearance because they contain shrunken endosperm. When the pollen of the first strain (P_1) is dusted on the silks of the second (P_1) (or vice versa), the kernels produced (F_1) are all yellow and full. This tells us that the genes for color (C) and fullness (S) are dominant over those for colorlessness (c) and shrunken endosperm (s) (Fig. 18.12). The plants grown from the F_1 kernels are then crossed with the homozygous recessive (P_1) strain (*ccss*) to find out what kinds of gametes are produced by the F_1 plants. According to Mendel's second law, the inheritance of the genes determining color should be entirely independent of the inheritance of the genes determining the appearance of the endosperm. The heterozygous F_1 should thus produce the following gametes in approximately equal numbers: (1) *CS,* as inherited from one parent, (2), *cs* as inherited from the other parent, (3) *Cs,* a recombinant, and (4) *cS,* a recombinant. All the gametes produced by the double homozygous recessive in the cross would be *cs.*

Linked genes tend to segregate together because they occur on the same chromosome

Random union of these gametes would result in four kinds of individuals: (1) colored, smooth, as in the original strain, (2) colorless, wrinkled, as in the other original strain, (3) colored, wrinkled, a recombinant, and (4) colorless, smooth, another recombinant. The genetics of this cross and the actual results achieved are shown in Fig. 18.12. Obviously, Mendel's *law of independent assortment* does not hold. There is a very strong tendency for the gene for colored (C) to remain associated with the gene for smooth texture (S), and similarly, for the gene c to remain associated with the gene s. As we have seen, it can be explained by assuming that the loci of the genes controlling kernel color are on the same chromosome as the loci of the genes controlling kernel texture. In the F_1 generation, when the chromo-

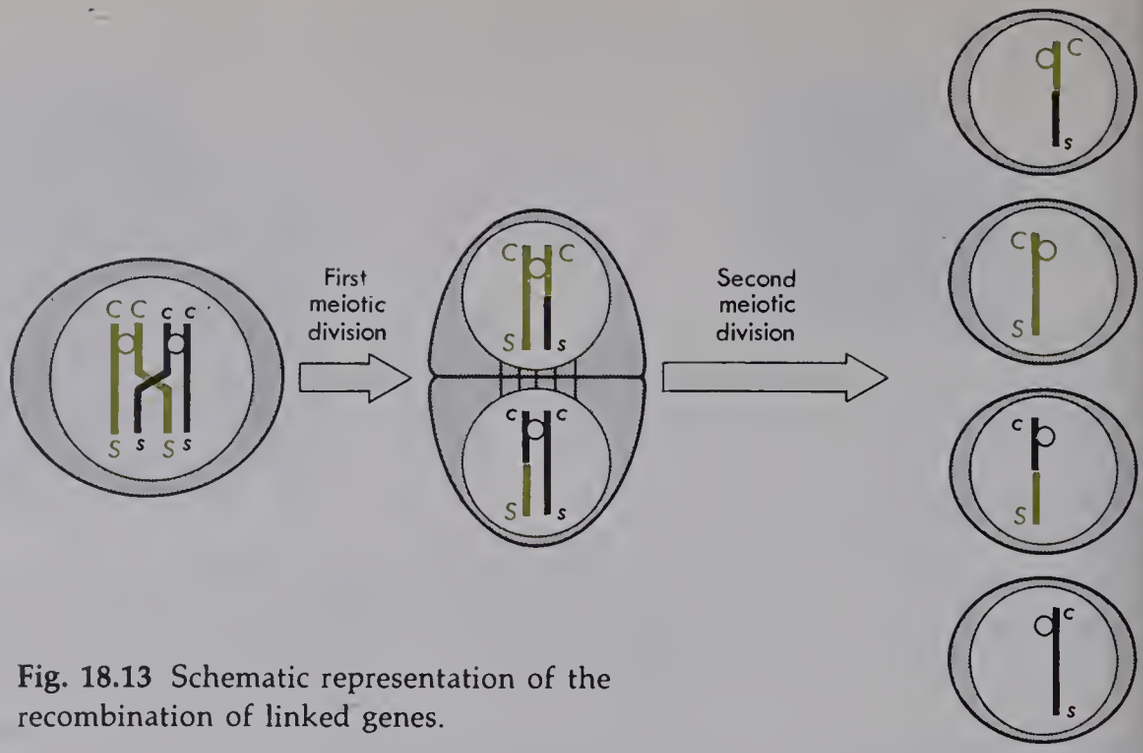


Fig. 18.13 Schematic representation of the recombination of linked genes.

somes separate in the first meiotic division, the paternal chromosome (*CS*) goes to one pole and the maternal chromosome (*cs*) goes to the other (Fig. 18.13).

Linked genes become separated when crossing over occurs between them

One question remains. If the gene loci for color and texture are on the same chromosome, how can one explain the 301 recombinant individuals (3.6%) produced by the testcross? The answer is clear when one remembers precisely what happens in the prophase of the first meiotic division. Pairs of duplicated homologous chromosomes unite in synapsis and then one chromosome of each homologue exchanges one or more segments in the process of "crossing over." It is this process of crossing over that gives rise to recombinant gametes. In this case, whenever the crossover occurs between the gene locus for kernel color and the gene locus for kernel texture the original combination of alleles (*CS* and *cs*) will be broken up and a chromosome containing *Cs* and one containing *cS* will be produced. Note, however, that the other 2 homologues do not participate in this process. Thus only one-half of the gametes produced after second meiosis will contain the recombinant genotype. Remembering that 3.6% of the total gametes produced by the F_1 generation were recombinants, in what percent of the meiotic divisions did crossing over occur between the *C* and *S* loci? (The answer is 7.2%. Do you see why?)

18.6 CHROMOSOME MAPS

Other linked traits have been studied in corn. In some, the percentage of recombinant gametes formed by F_1 individuals is even less than the 3.6% found above. In most, the percentage of recombinant gametes is higher. Assuming for a moment that the gene loci are in a linear order from one

The higher the percentage of recombinant gametes, the greater the distance separating the loci

end of a chromosome to the other, we may deduce that the higher the percentage of recombinant gametes formed for a given pair of traits, the greater the distance separating the two loci. The opposite is also true. Using this knowledge, one can plot a linkage map for as many of the gene loci on a given chromosome as can be discovered. For example, we have seen that about 3% recombinant gametes are produced as a result of crossing over between gene loci *C* and *S*. A dihybrid can then be developed for another pair of linked traits, say kernel color (*C*) and waxy endosperm (*wx*). This dihybrid produces 33% recombinant gametes, indicating that the loci *C* and *wx* are farther apart than the loci *C* and *S*. However, one does not yet know whether the locus *wx* is on the same side of *C* as *S* or on the opposite side (Fig. 18.14). The answer can be determined by developing a dihybrid for *S* and *wx*. If the percentage of recombinant gametes produced by this individual is less than 33%, then the gene locus *wx* must be on the same side of locus *C* as locus *S*. The opposite is true if the percentage of recombinant gametes turns out to be greater than 33%. Actually the number of recombinant gametes formed is about 30%. Thus we know that the sequence of gene loci on this chromosome is *C-S-wx*. Furthermore, the fact that the sum of the percents of recombinants between *C* and *S* and *S* and *wx* is so close to the percent between *C* and *wx* lends strong support to the idea that the gene loci are arranged in a line along the length of the chromosome (Fig. 18.14). A straight line is the only geometric arrangement in which this simple numerical relationship can exist.

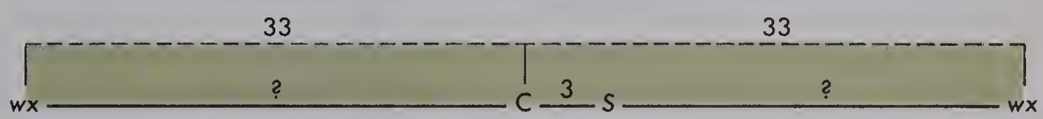


Fig. 18.14 Plotting a linkage map. The production of 30% recombinant gametes as a result of crossing over between the *S* and *wx* loci tells us that locus *wx* is on the same side of locus *C* as is locus *S*.

By pursuing this method with as many linked genes on a given chromosome as can be discovered, it is possible to plot chromosome maps. These maps show the sequence in which the gene loci occur and the relative spacing between them. Such maps have been produced for the chromosomes of the fruit fly (Fig. 18.15), corn, certain bacteria, the mouse, the silkworm, the tomato, *Chlamydomonas,* and the human X chromosome.

Linkage groups

As geneticists have established which genes in a particular organism show linkage and which do not, they have been able to assign each gene to a *linkage group.* All the genes in a given group show linkage with respect to each other. They do not show linkage to genes in other groups. The interesting thing about this is that the *number* of linkage groups in an organism is equal to the number of homologous pairs of chromosomes in that organism. Thus *Drosophila melanogaster,* with its four pairs of chromo-

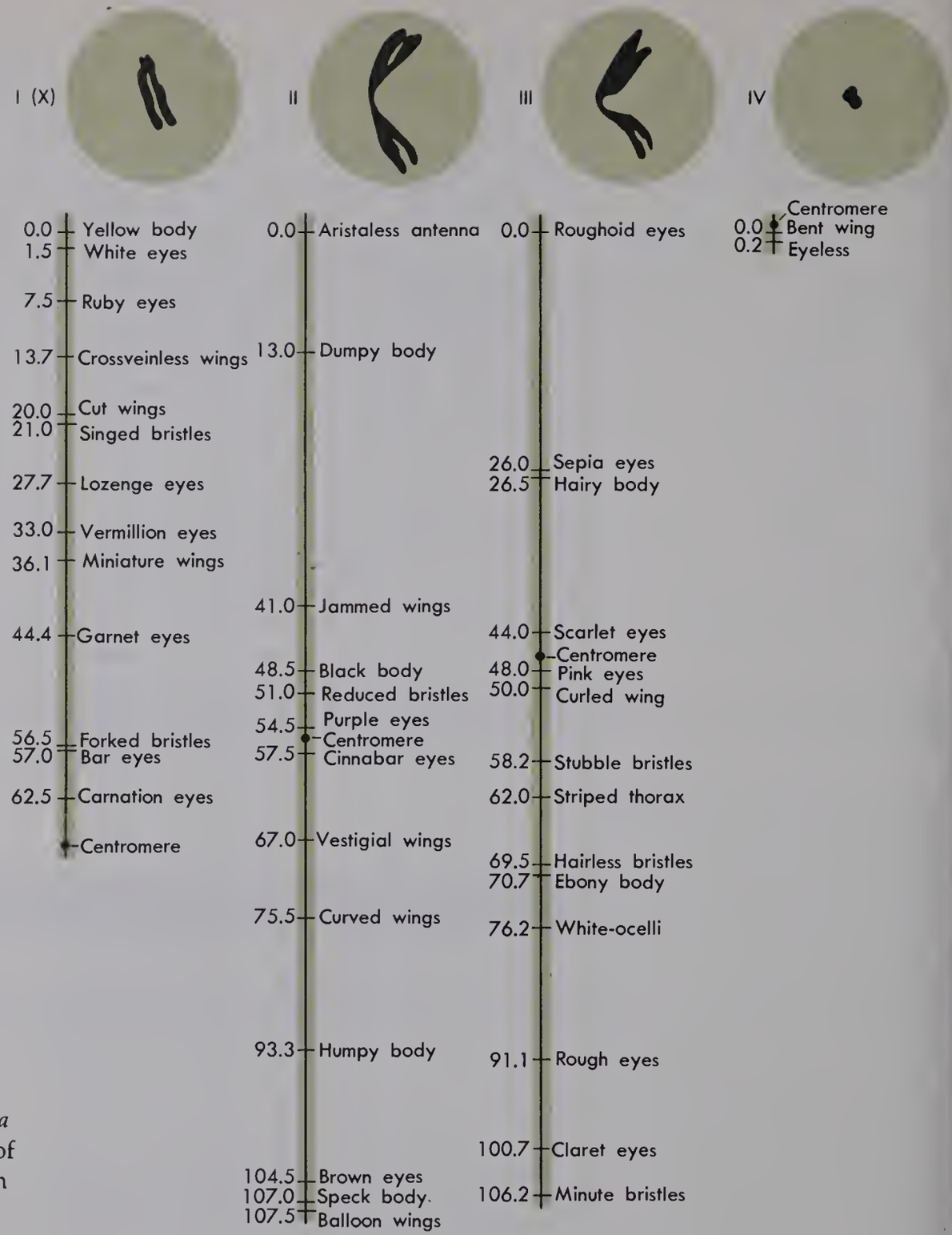


Fig. 18.15 Maps of the chromosomes of *Drosophila melanogaster.* Only a few of the many gene loci known are listed.

somes, has four linkage groups. The corn plant has 10 pairs of chromosomes. All of its *genes* fall into one or another of 10 linkage groups. Similarly, in *Neurospora* and the tomato plant, both of which have been exhaustively studied by geneticists, the number of linkage groups is equal to the number of pairs of chromosomes—7 in *Neurospora* and 12 in the tomato. Here, then, is additional evidence that an organism's genes are located in or on the chromosomes in the cells of that organism.

18.7 THE EVIDENCE OF CREIGHTON AND McCLINTOCK

Creighton and McClintock showed that recombination of traits accompanies crossing over

Perhaps the most elegant demonstration of the chromosome theory came in 1932 from the work of geneticists Harriet Creighton and Barbara McClintock. During the course of their studies of linkage in corn, they developed a strain of corn that had one chromosome (out of 10 pairs) with two unusual features: a knob at one end of the chromosome and an extra piece of chromosome on the other. This extra piece of chromosome was the result of a translocation that had occurred in an earlier generation. These workers saw immediately the use to which their discovery could be put. Here was an organism with a pair of homologous chromosomes which could easily be distinguished from each other by microscopic examination. Furthermore, this unusual chromosome carried the dominant allele for colored kernels (*C*) and the recessive allele for waxy endosperm (*wx*). Its normal-appearing mate carried the recessive allele for colorless kernels (*c*) and the dominant allele for normal (starchy) endosperm (*Wx*). Thus the plant was a dihybrid for these two linked traits and, in addition, one chromosome of the pair was visibly marked at each end. Creighton and McClintock reasoned that this plant would produce four kinds of gametes: the parental kinds (*Cwx* and *cWx*) and the recombinant kinds (*cwx* and *CWx*) produced by crossing over. Fertilization of these gametes by gametes containing a chromosome of normal appearance and both recessive alleles *cwx* (a typical testcross) should produce four kinds of kernels: (1) colored waxy (*Ccwxwx*) kernels, (2) colorless kernels with normal endosperm (*ccWxwx*) and the two recombinant types, (3) colorless waxy (*ccwxwx*), and (4) colored kernels with normal endosperm (*CcWxwx*). Furthermore, mi-

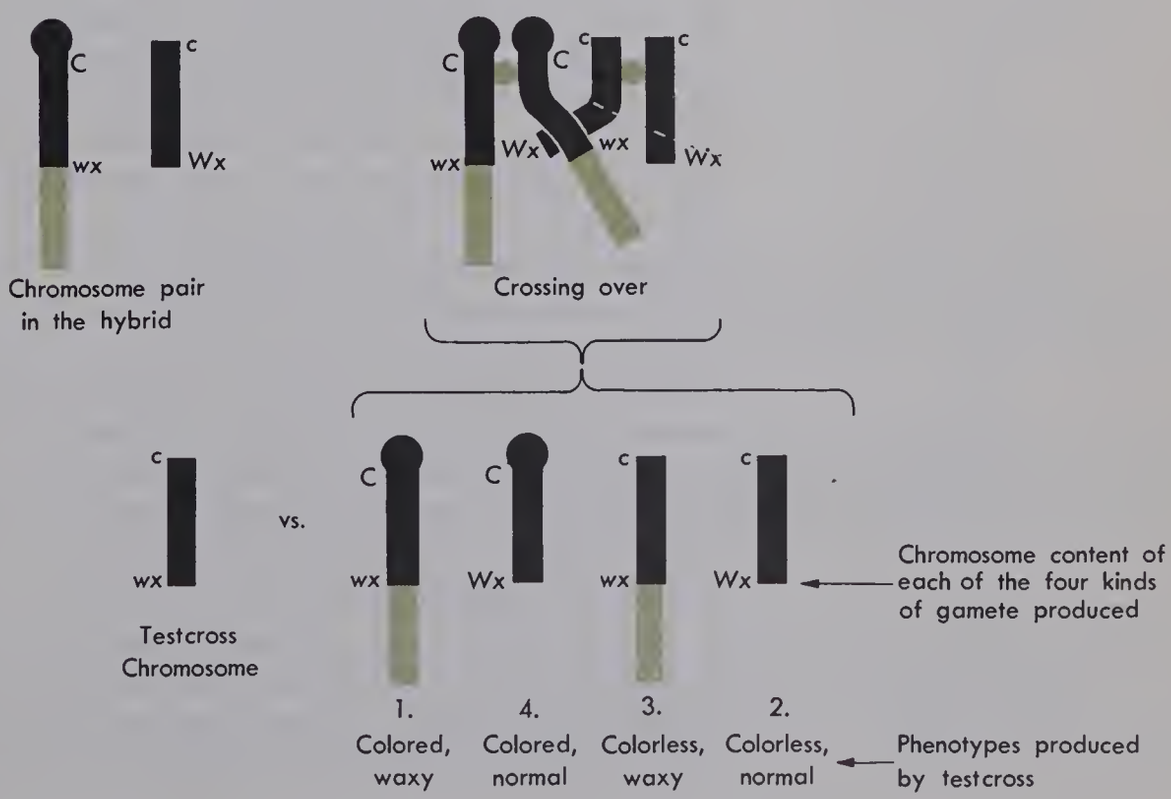
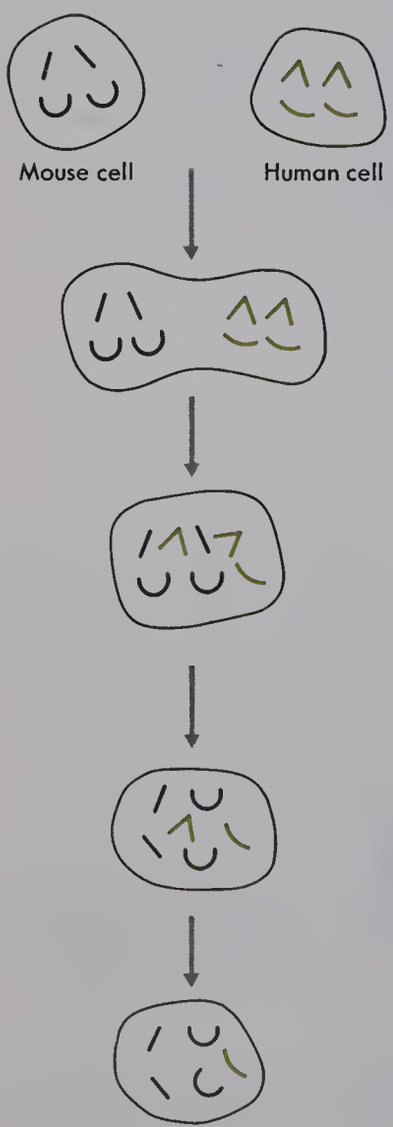


Fig. 18.16 Correlation of cytological crossing over and genetic recombination in corn.

croscopic examination of each of the plants resulting from these four kinds of kernels should reveal the following kinds of chromosomes. In the first case, there should be one normal chromosome and one extra-long chromosome with the knob at the end. In the second case, both chromosomes should be of normal appearance. However, in the third case, where crossing over had occurred, one would hope to find evidence that a physical exchange of parts between the homologous chromosomes of the dihybrid parent had occurred. Either a chromosome of normal length but with a knob at one end should be present or an extra-long chromosome with no knob. Actually Creighton and McClintock found the latter, thus indicating that the gene locus for *wx* was associated with (and thus near) the end of the chromosome with the extra segment. The gene locus for kernel color must then be nearer the end with the knob. Examination of the plants in Class 4 (colored kernels-*C* and normal endosperm-*Wx*) revealed a chromosome of normal length but with a knob at one end (Fig. 18.16).

Thus, behavior of the genes as revealed by study of the phenotypes produced was shown to be directly related to the behavior of the chromosomes as revealed by microscopic examination. The recombination of genes is now shown to occur at the same time that homologous chromosomes exchange parts. This is a very convincing demonstration of the chromosome theory. The determinants of the hereditary traits, genes, whose existence was first hypothesized by Mendel, are shown indeed to be located in these visible structures, the chromosomes.



18.8 ASSIGNING LINKAGE GROUPS TO CHROMOSOMES

If an investigator can establish a linkage group and can assign one gene in it to a particular pair of homologous chromosomes, he immediately has a list of traits controlled by those chromosomes. The remarkable materials with which Creighton and McClintock worked enabled them to assign all the known genes of linkage group IX to one pair of the corn plant's 10 pairs of homologous chromosomes. They were able to do this because they could associate the inheritance of a particular gene with the inheritance of a chromosome that could be visually distinguished from all the others (including its homologue). Their success thus depended upon the discovery of an abnormal chromosome and the ability to breed the plant containing it as they wished. A few associations between the inheritance of a visually distinguishable (and hence abnormal) chromosome and the inheritance of a particular trait have recently been discovered in humans. Other approaches have, however, been more fruitful in enabling us to assign known genes to particular chromosomes. One approach, which is beginning to produce useful information, depends upon the technique of somatic cell hybridization.

Human cells grown in tissue culture can be made to fuse with cells from other animals such as the mouse. At first, such hybrid cells contain a complete set of both human and mouse chromosomes. However, as mitosis continues, many of the human chromosomes become lost. Now,

human cells possess a number of biochemical features, e.g. particular enzymes, not shared by mouse cells. If every time a particular human enzyme is lost in a clone of hybrid cells, a particular human *chromosome* is lost as well, we may assume that the gene controlling the synthesis of that enzyme is located on that chromosome. We may also assume that every other gene in the linkage group to which that gene is assigned is also on the chromosome. For example, mouse-human hybrid cells that produce a particular human peptidase always retain a number 1 human chromosome. Conversely, no mouse-human hybrid clones that lack the human number 1 chromosome produce the human peptidase. Some nine human genes (including those that specify the Rh blood factor) have been shown through family studies to be linked to the peptidase gene. Thus we conclude that all these genes are present on the number 1 chromosomes. A partial list of recent gene-chromosome assignments in humans is given in Fig. 18.17. By the time you read this, the list will probably be much longer.

Fig. 18.17 The chromosome assignments of a number of human genes. With the exception of those that are sex-linked, the majority of these genes have been assigned through the analysis of somatic cell hybrids. Once a single member of a linkage group can be assigned to a particular chromosome, all the other genes in that linkage group receive the same assignment.

Chromosome	Genes
1	Peptidase C, Rh factor, salivary amylase
2	α chain of hemoglobin?
4 or 5	β chain of hemoglobin?
7	Mannose phosphate isomerase
11	Lactate dehydrogenase A
12	Lactate dehydrogenase B, peptidase B
X	Clotting factor VIII (absence produces hemophilia), over 80 others
14	Nucleoside phosphorylase
15	ABO blood group antigens
16	Adenine phosphoribosyl transferase
17	Thymidine kinase
18	Peptidase A
19	Glucose phosphate isomerase
20	Certain transplantation antigens?

EXERCISES AND PROBLEMS

1. The garden pea has seven pairs of homologous chromosomes. If Mendel had mated all possible dihybrid combinations of eight pairs of contrasting traits instead of seven, would his *law of independent assortment* still have held? Explain.
2. In man, normal vision is a sex-linked trait and its gene is dominant to the allele for red-green colorblindness. When a color-blind woman marries a man with normal vision, what would be the expected distribution of color vision among (a) their sons and (b) their daughters?
3. How many pairs of homologous chromosomes are found in the human male?
4. Summarize the parallelism between the behavior of genes as described by Mendel and the behavior of chromosomes in meiosis and fertilization.
5. Nondisjunction may lead to zygotes containing *XXY*. What sex would this produce in a fruit fly? In a human?

6. When tall tomatoes with red fruit are crossed with dwarf tomatoes with yellow fruit, all the offspring are tall-red. When these F_1's are mated, only the two P_1 phenotypes are found in the F_2. What do we conclude about the genetic control of these two traits?
7. Define a gene.
8. For some reason (not yet fully understood) more male babies are conceived than female. By childhood, the number of boys and girls is the same. Can you think of a possible explanation for the higher mortality (both before birth and after) of male babies?
9. By testcrossing the following dihybrids, it is shown that each produces recombinant gametes in the percentage indicated.

AaBb	31%	AaXx	36%
XxYy	22%	AaYy	14%
XxBb	5%		

   From these data determine the linear sequence and relative spacing of each gene locus on the chromosome.
10. Predict the sex of humans with the following chromosome abnormalities (all of which have been observed): *XXY, XXX, X, XYY, XXXX, XXXY, XXXXY.*
11. How many Barr bodies would you expect to find in the cells of each of the individuals in the previous problem?

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19

THE NATURE AND ACTION OF GENES

The Chemical Nature of the Genes

The Watson-Crick Model of DNA

Mutations

The One Gene–One Enzyme Theory

Protein Synthesis

The Code

Verification of the Code

Multiple Alleles

Reverse Transcriptase

The Prospects of Genetic Engineering

The Action of the Total Genotype

The idea (though not the name) of the gene was developed by Mendel in an attempt to explain certain rules of inheritance. Mendel made no attempt to visualize the gene as a specific structure, in a specific location, with a specific chemical nature, and a specific method of action. Once it was fully established that the genes are located on the chromosomes, however, it became possible to make this attempt. Chromosomes are visible structures in a specific location. It is possible to isolate them from the rest of the cell and to study their chemical composition.

19.1 THE CHEMICAL NATURE OF THE GENES

Chromosomes contain DNA and protein

Chromosomes are made of nucleoprotein. By gentle chemical treatment it is possible to break nucleoprotein into its constituent parts: protein and nucleic acid. The chemical nature of each was discussed in Chapter 4. You remember that proteins are macromolecules made up of 20 kinds of repeating units called amino acids. Although all proteins have this same basic structure, the sequence of amino acids is almost infinitely variable. Thus it is not surprising that proteins from different species, proteins from different individuals within a species, and proteins from different locations within the individual all show physical and chemical differences.

Nucleic acids are also macromolecules, and they, too, have a basic structure of repeating units. These units are of three types: bases, sugars, and phosphate groups. The nucleic acid found in chromosomes is DNA. Its bases are generally restricted to four kinds: two purines (adenine and guanine) and two pyrimidines (thymine and cytosine). For convenience, we will henceforth refer to these as A, G, T, and C, respectively. The sugar is deoxyribose and it supplies the name, *d*eoxyribo*n*ucleic *a*cid, for DNA.

DNA

RNA

A second nucleic acid, *r*ibo*n*ucleic *a*cid (RNA), is found chiefly outside of the nucleus. In RNA, ribose is the sugar unit rather than deoxyribose. Ribose differs from deoxyribose in having one more oxygen atom in its molecule. Another pyrimidine, uracil (U), substitutes for thymine in RNA.

Now that we know that chromosomes are made up of two kinds of macromolecules, protein and DNA, the question is whether the hereditary information is carried by one or both of these substances. In 1928, an English bacteriologist, Fred Griffith, made a discovery which, pursued over the next 30 years, ultimately revealed the answer. Griffith worked with the bacterium that causes bacterial pneumonia, a widespread and dangerous disease before the discovery of antibiotics. One of the most striking features of this organism is the presence around each cell of a gummy capsule made of a polysaccharide. When these bacteria are grown in culture, the presence of the capsule causes the colonies to have a glistening, smooth appearance (Fig. 19.1). Because of this, the cells are referred to as "S" cells.

"Smooth" and "rough" pneumococci

However, after prolonged cultivation outside the living host, some cells lose the ability to make the capsule. The surface of their colonies then appears wrinkled and rough ("R") (Fig. 19.1). With the loss of the ability to make the capsule, the organism also loses its virulence. The R forms cannot cause the disease.

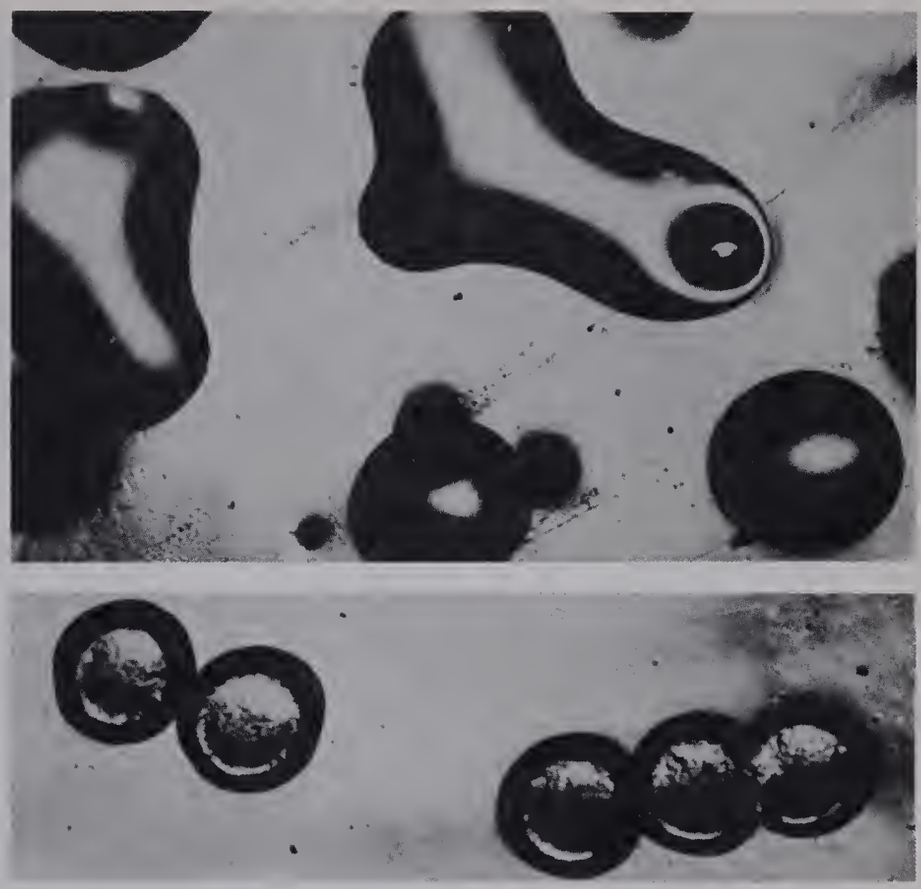


Fig. 19.1 Pneumococci growing as colonies on the surface of a culture medium. Top: the presence of a capsule around the bacterial cells gives the colonies a glistening, smooth (S) appearance. Bottom: pneumococci lacking capsules have produced these rough (R) colonies. (Research photograph of Dr. Harriett Ephrussi-Taylor, courtesy of The Rockefeller University and *Scientific American.*)

Types of pneumococci

Pneumococci, as these bacteria are known, also occur in a large variety of types: I, II, III, etc. Each type produces its own specific polysaccharide capsule, which can be distinguished from that produced by other types. Unlike the occasional shift of S $\longrightarrow$ R, the type of the organism is constant. Mice injected with a few cells of one type, say II, will soon have their bodies teeming with descendant cells of the same type. With respect to type, then, we find the same continuity of traits from generation to generation that we found in other organisms when they reproduce asexually.

When injected alone, neither living R cells nor S cells that have first been killed by subjecting them to a high temperature cause any symptoms of disease. Griffith unexpectedly found, however, that when living R cells and killed S cells were injected *together* into a mouse, the mouse became ill and *living* S cells could be recovered from its body. In exploring this phenomenon further, Griffith discovered that the type of the S cells was determined by the type of the dead S cells he used, not the type of the strain from which the living R cells were derived. In other words, when a mouse was injected with living R cells from Type I pneumococci and dead S cells of Type II, the living cells that were recovered after the mouse became sick were S cells of Type II. Furthermore, this change of R-I cells to S-II cells was stable and inheritable. The S-II cells could be cultured indefinitely and remained true to type. Clearly, something in the dead S-II cells had converted the R-I cells into S-II cells, and that something was passed on from generation to generation.

Griffith discovered transformation



Fig. 19.2 Electron micrograph of a DNA molecule entering a pneumococcus (the large object at the right). This DNA molecule, the long fine line, is approximately 7 micrometers (μm) in length, long enough to include a dozen genes. The process of *transformation* follows the uptake of such a molecule by the bacterium. (38,000 X, courtesy of Dr. Alexander Tomasz.)

Within a few years, this *transformation,* as it was called, was accomplished in a test tube. A small percentage of R-I cells, grown in test tubes of broth to which *dead* S-II cells were added, became transformed into S-II cells. Later, a cell-free extract of S-II cells was successfully used to transform R-I cells into S-II cells.

As you would expect, this cell extract had a variety of ingredients in it, including polysaccharides, protein, DNA, RNA, and lipids. By selectively destroying one of these ingredients after another and testing the transforming activity of the remaining material, Dr. O. T. Avery and his coworkers were able to show in 1943 that the active ingredient of the transforming principle was DNA (Fig. 19.2). Purified DNA from S-II cells transformed R-I cells into S-II cells. Here, then, was a molecule which changed the function of the recipient cell, making it produce, in this case, a type II rather than a type I capsule. Furthermore, this molecule was self-duplicating. Far more S-II-transforming DNA could be extracted from the offspring of the transformed cells than was used originally to accomplish the transformation.

Transformation is accomplished by DNA

In 1952, geneticists A. D. Hershey and Martha Chase performed a series of experiments with viruses that gave further proof that DNA is the basic hereditary material. In many ways viruses resemble small chromosomes. They, too, are made of nucleoprotein and when they enter a host cell, the latter goes to work duplicating the virus particles. It is as if the normal genetic control of the cell has been taken over by foreign, invading genes. Bacteria may be infected with viruses called **bacteriophages.** The process of entry has been observed under the electron microscope. Only an inner portion of the virus particle actually enters the cell (Fig. 19.3). The exterior part, the virus coat, probably serves to inject the infecting portion.

Bacteriophages are bacterial viruses

Bacteriophages produced within bacteria growing in radioactive culture medium will themselves be radioactive. If radioactive sulfur atoms are present in the medium, they will be incorporated into the proteins of the bacteriophage, since two of the twenty amino acids found in proteins contain sulfur atoms. There are no sulfur atoms in DNA. On the other hand, radioactive phosphorous atoms will be incorporated into the DNA

The experiments of Hershey and Chase



exclusively, for it alone contains phosphorus atoms. When bacteriophages containing radioactive phosphorous atoms are allowed to infect *non*radioactive bacteria, all the bacterial cells become radioactive. Much of this radioactivity is then passed on to the next generation of bacteriophages. However, when bacteria are infected with viruses containing radioactive sulfur atoms and then the virus coats are removed (by whirling in the Waring blendor), practically no radioactivity can be detected in the cells. It is retained in the virus coats.

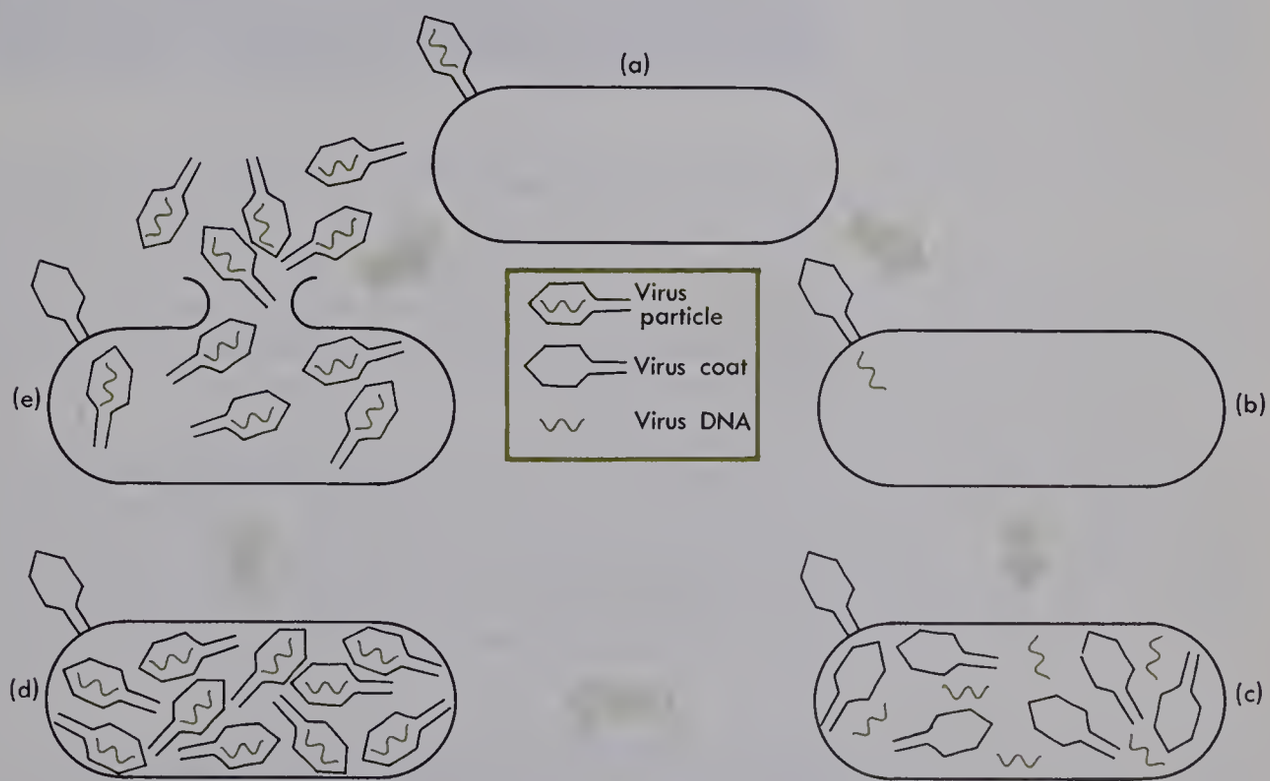


Fig. 19.3 Cycle of infection of bacterial cells by bacteriophages. After the virus attaches to an uninfected bacterial cell (a), its content of DNA is injected (b). New viral DNA molecules *and* new viral protein coats are produced (c). From these are assembled infective bacteriophages (d), which are released when the bacterial cell bursts (e).

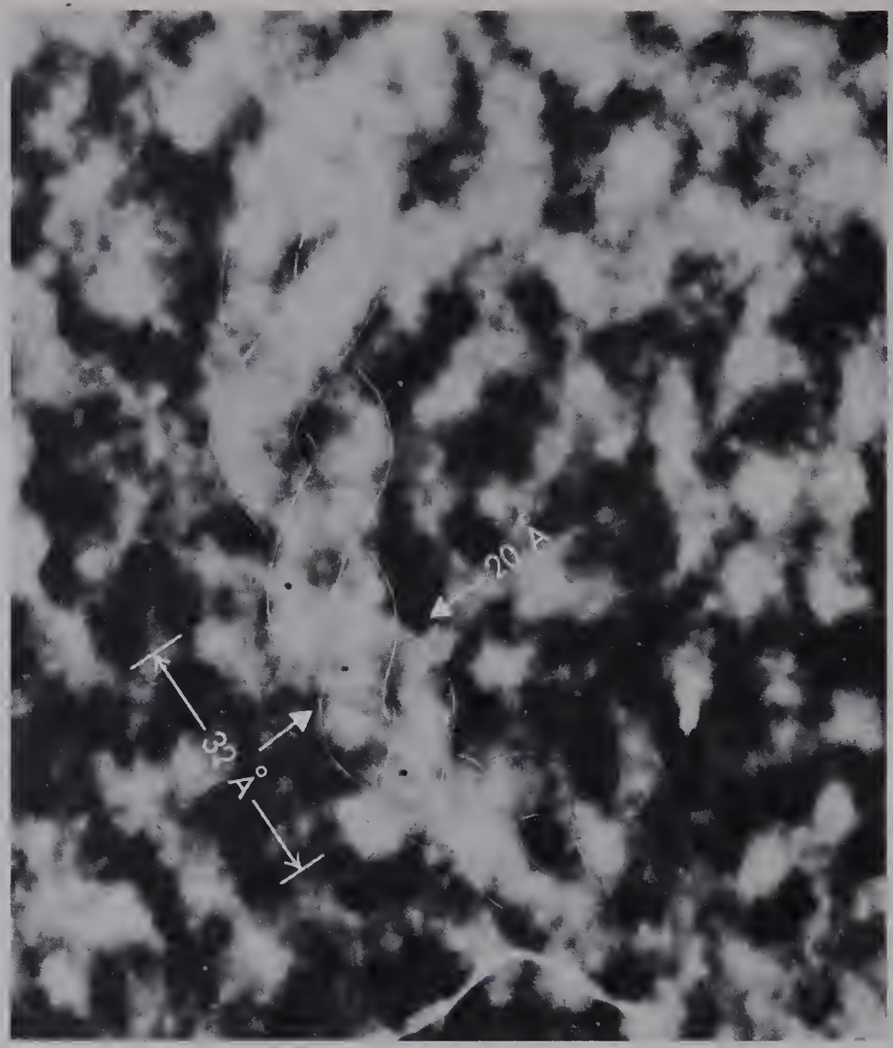


Fig. 19.4 Direct visualization of the helical structure of DNA. Lines have been added to the electron micrograph to delineate the two strands of the molecule more clearly. (Courtesy of Dr. Jack Griffith, Stanford Medical School.)

From these experiments we conclude that the DNA component of the bacteriophage is injected into the bacterial cell while the protein component remains outside. The DNA is, however, able to direct the formation of new virus particles *complete* with protein coats. Clearly, DNA is the fundamental hereditary material, the substance of the genes. To understand how DNA carries out this function, it is necessary to know more about its chemistry.

A=T; C=G

In attempting to unravel the chemical nature of DNA, biochemists soon discovered an interesting fact about the relative amounts of the four bases that are present in it. Whatever the amount of A present (and it varies widely from species to species), the amount of T is precisely equal to it. Similarly, the amount of G present is always equal to the amount of C.

19.2 THE WATSON-CRICK MODEL OF DNA

It was the model proposed by Nobel prize winners James Watson and Francis Crick that first provided an idea as to the way these various constituents of DNA are attached to one another. They deduced that the sugar and phosphate groups alternate with one another in a long strand. Two of

these strands are twisted around each other in the form of a double helix, something like a double "spiral" staircase (Fig. 19.4). A base is attached to each sugar in the chain so that it projects in toward the axis of the staircase. There it joins with the base projecting from the other staircase. Because of the shape and chemical nature of the purine and pyrimidine bases, A can be attached only to T. Similarly, G can be attached only to C. This explains why the amounts of A and T (also C and G) in the molecule are always equal. Single DNA molecules may contain from hundreds of thousands to millions of base pairs.

Information can be encoded in DNA

The Watson-Crick model provides a clue to the manner in which hereditary information can be stored in the DNA molecule. Although the basic arrangement of parts in the DNA molecule seems to be the same for all organisms, there is an almost infinite variety of sequences in which these four kinds of base pairs, present by the hundreds of thousands, can be arranged. The International Morse Code provides an interesting parallel. It is made up of only three basic units: a dot, a dash, and a pause. Given sufficient patience and time, however, a telegraph operator could transmit the contents of an entire library by the sequences in which he combined these three units!

The two strands of the DNA molecule are complementary

At one step on a single staircase, any one of the four bases may be present. Thus, there are four items in our code. Note, too, that whatever base is present at a given step, we know immediately what kind of base bridges the gap between it and the opposite staircase. Thus whatever sequence of bases is present on one strand of the DNA molecule, a complementary sequence is present on the other strand. The two sequences have the same relationship to each other as the "positive" and "negative" of a photograph. This repetitious nature of the code provides a nice mechanism to explain one of the fundamental properties of genes: their ability to duplicate.

DNA replication

The chromosomes are duplicated during interphase, the period between cell divisions. According to the Watson-Crick hypothesis, the DNA molecules become "unzippered" at this time; that is, the bonds between the base pairs break and the two halves of the molecule unwind. Once exposed, the bases on each of the separated strands can pick up the appropriate nucleotides (a base linked with deoxyribose and phosphate) floating freely in the nucleus. Each exposed C will pick up a G, each G a C, etc. When the process is completed, two complete DNA molecules will be present, identical to each other and to the original molecule (Fig. 19.5). This is precisely the description we used in discussing what happens to chromosomes in the process of mitosis.

This attractive hypothesis has received considerable experimental support. As we saw in Chapter 16 (see Section 16.6), *E. coli* cells can be grown in a culture medium containing just glucose and inorganic salts. Among the latter must be a source of nitrogen atoms for protein and nucleic acid synthesis. Ammonium ions (NH_4^+) serve nicely. Although the most common isotope of nitrogen is ^{14}N, it is possible to synthesize NH_4^+



Fig. 19.5 The mechanism of duplication of DNA.

containing a heavier isotope of nitrogen, ^{15}N. When *E. coli* cells are grown for several generations in a medium containing $^{15}NH_4^+$, their DNA becomes about 1% heavier because of the incorporation of ^{15}N atoms in it. Working at Caltech in 1958, M. S. Meselson and F. W. Stahl grew *E. coli* cells in this way and then let them divide once in a medium containing ordinary ammonium ions, $^{14}NH_4^+$. The DNA in the new generation of bacterial cells was exactly intermediate in weight between the heavier DNA in the previous generation and the normal. This, in itself, is not surprising. It tells us no more than that half the nitrogen atoms in the new DNA are ^{14}N and half are ^{15}N. It tells us nothing about their arrangement in the molecule. However, when the bacterial cells were allowed to divide *again* in normal ammonium ion ($^{14}NH_4^+$), two distinct weights of DNA were formed: half the DNA was of normal weight and half was of intermediate weight. As shown in Fig. 19.6, this indicates that DNA molecules are not degraded and reformed at each interphase, but instead, each original strand remains intact as it builds a complementary strand from the nucleotides available to it.

The experiment of Meselson and Stahl

Taylor's experiment

Watson and Crick's theory of DNA duplication is reflected even at the level of the visible chromosomes. When plant root tips are grown in a solution containing radioactive thymidine (the nucleotide containing thymine, T), the chromosomes in the rapidly dividing cells of the embryonic region become uniformly radioactive. If the root tips are then transferred from the radioactive solution to one containing nonradioactive thymidine and colchicine, chromosome duplication takes place without cell division. After one duplication, the chromosomes are still uniformly radioactive.

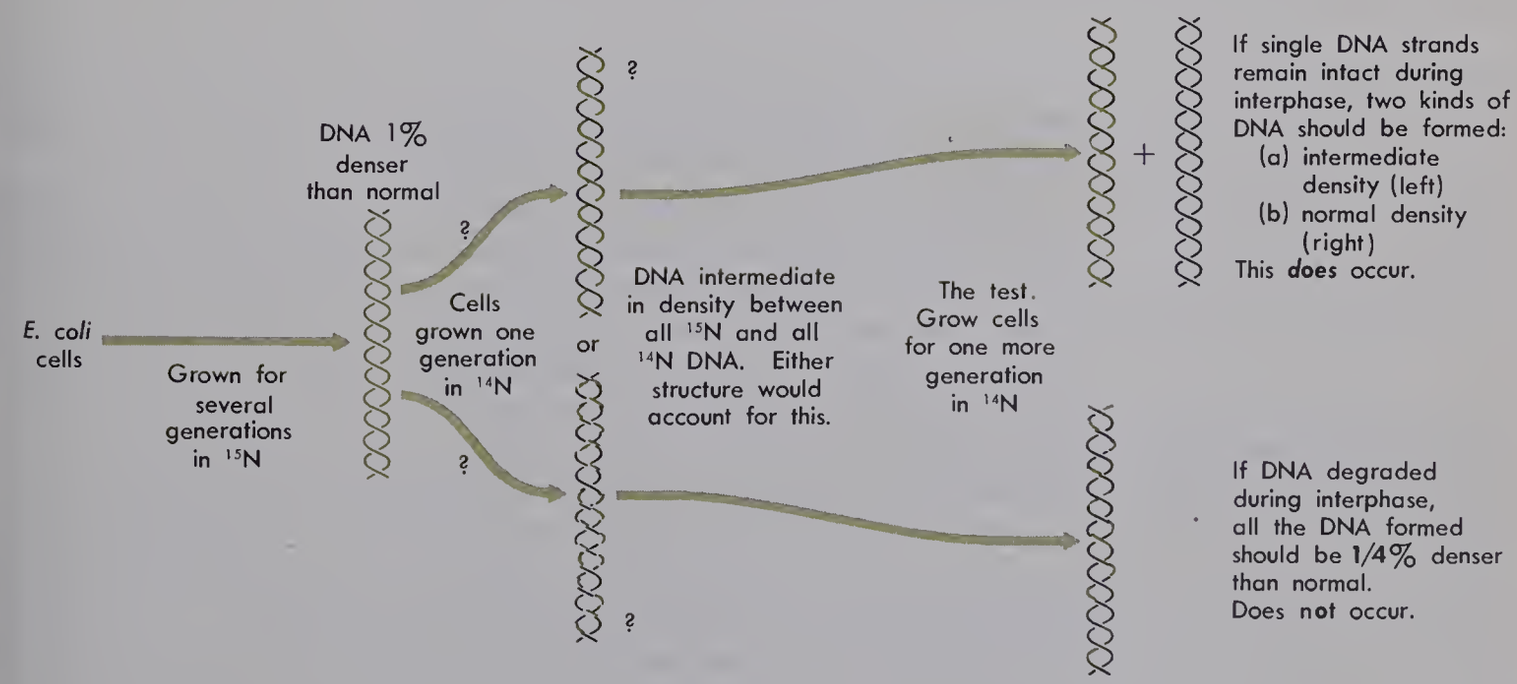


Fig. 19.6 The experiment of Meselson and Stahl. The results (right) suggest that during the process of duplication each strand of DNA remains intact and builds a complementary strand from the nucleotides available. This is consistent with the Watson-Crick model of DNA.

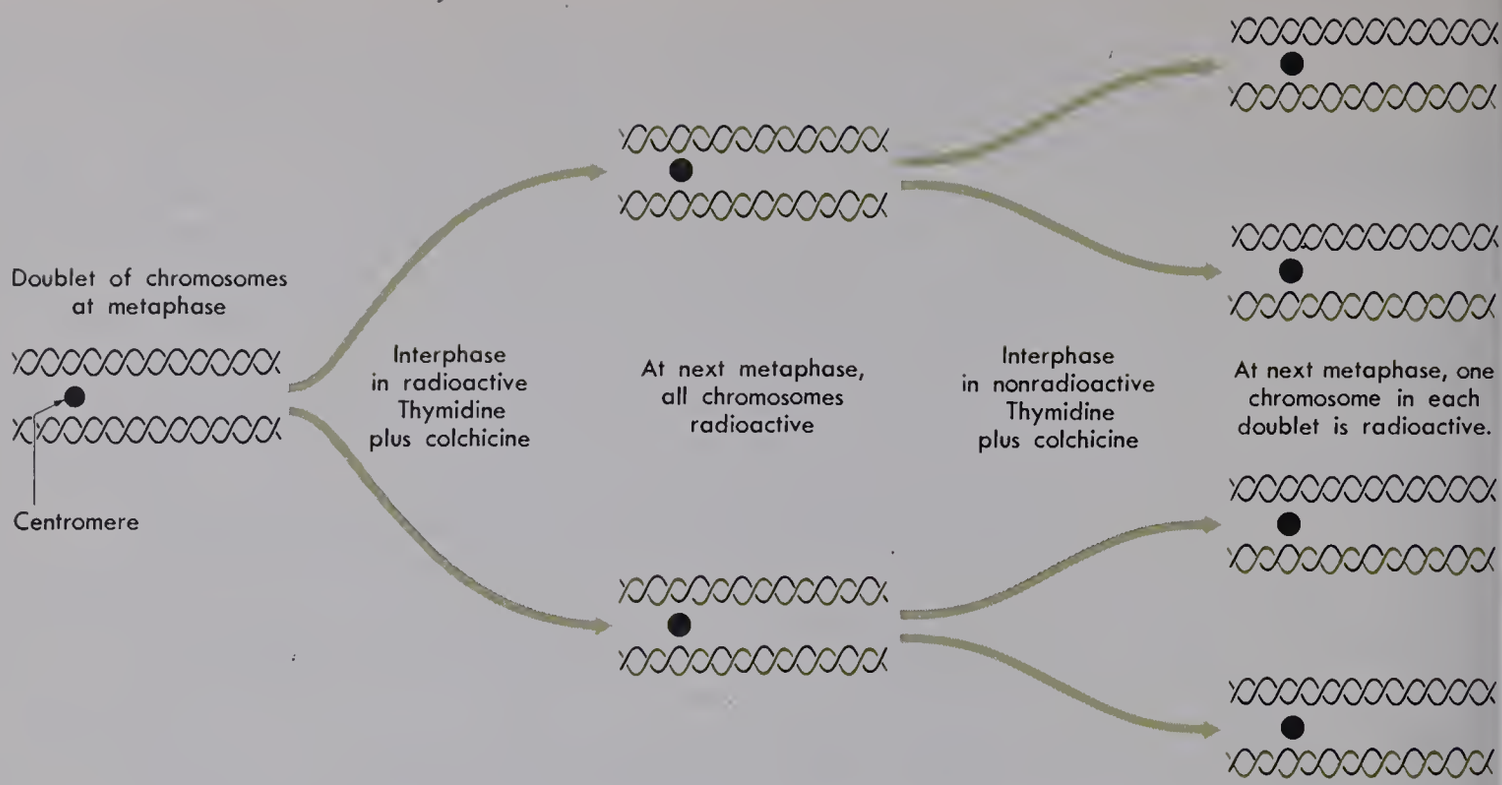


Fig. 19.7 The duplication of chromosomes. The results suggest that (1) each chromosome (chromatid) consists of *two* parts and (2) each of these two parts remains intact during the process of duplication. The parallel between this experiment and the one shown in Fig. 19.6 suggests that the two parts are the two strands of a DNA molecule and that they are separated during interphase according to the Watson-Crick model. Exactly how the DNA is organized in the chromosome is not yet known. This experiment was first performed by Dr. J. Herbert Taylor.

After a second duplication, however, one chromosome of each doublet is radioactive, the other is not (Fig. 19.7).

Thorough analysis of the genetics of *E. coli* has made it abundantly clear that all the genes of this organism are incorporated in a single molecule of DNA almost 1 mm long (although only 20 A in diameter!). Such a molecule has actually been isolated from *E. coli* cells. "Sprung loose" might be a better expression, since this one-millimeter *molecule* is folded up in a *cell* that is only about 2 μm long. In *E. coli,* therefore, the chromosome of the organism is simply a DNA molecule. The chromosomes of higher organisms are far larger and more complex in their organization. In fact, it is not yet known whether a single chromosome in a higher organism contains one molecule of DNA (as you might expect from Taylor's evidence—Fig. 19.7) or whether it contains many of them.

It is not yet known just how the DNA is organized in the chromosomes of eukaryotes

The mechanism for the duplication of DNA must be remarkably precise. Genes may be passed on for thousands of generations without showing any change. Today certain species of organisms are found which are called living fossils because they are so little changed from fossil forms living millions of years ago. The mollusk *Neopilina* (Fig. 1.2), living in the

The stability of genetic information

ocean today, is little changed from *Pilina* which lived in the oceans over 500 million years ago. When you consider that our Rocky Mountains were formed 60 million years ago, you can appreciate the great precision by which this particular hereditary information has been passed on from generation to generation.

The structure of the DNA molecule is ideally suited to the long-term preservation of the sequence of bases coded in it. Because the two chains in a DNA molecule are complementary, the information in one is also coded in the other. In a sense, then, each DNA molecule contains two copies of its information. If for some reason the sequence of bases on one chain were disturbed, the correct message could still be determined from the complementary chain. Recently it has been discovered that the cells of some bacteria and higher organisms (including ourselves) have a mechanism by which they correct errors that occur in their DNA code. By means of enzymes, they remove damaged or incorrect bases from their DNA molecule. These are then replaced by the correct bases, that is, bases complementary to the bases on the opposite strand. In this way, their genetic code is preserved intact.

The repair of DNA

19.3 MUTATIONS

Although the organization of the DNA molecule makes it remarkably resistant to changes in its sequence of bases, such changes do occur. These changes we call gene or "point" mutations. They may occur in the body cells of an organism. In this case, the welfare of the species is little affected. The mutant gene may cause the cell to age or die or perhaps to become cancerous, but when the owner of the cell dies, the mutant gene dies with him.

Point mutations

On the other hand, mutations that occur in the gamete-producing cells may be of great significance. We have seen how a mutation that probably occurred in the gonads of Queen Victoria's father gave rise to genes which produced many hemophiliacs in the royal families of Europe.

The mutant gene for hemophilia illustrates two points about most mutations. They are usually recessive and they are usually harmful. Why this is so will be clear when we discuss the mechanism of evolution in Chapter 32.

Mutations such as the one that first appeared in Queen Victoria are called "spontaneous." This is another way of saying that we don't know what caused the change in the genetic code. But methods have been found to induce mutations. In 1927, H. J. Muller reported that he had increased by 15 times the rate of mutations in *Drosophila* by treating them with x-rays. Muller ultimately received the Nobel Prize for this work. Since that time other radiations of short wavelength such as ultraviolet rays and cosmic rays have been found to induce mutations when they penetrate to the chromosomes. High temperatures and certain chemical substances, such as the nitrogen-mustard war gases, also produce mutations indistinguishable from those produced by radiation.

X-rays increase the mutation rate

Mutations are of great importance and interest to the biologist and the layman alike. First, the biologist sees mutations as the basic source of the variability among living things that has permitted evolution to occur. Second, the biologist recognizes that the vast majority of mutations produce harmful effects on the organism. These harmful genes may be passed on to future generations, carrying with them their burden of illness and deformity. The biologist also sees that man, as he develops his technological civilization, is exposing himself to increasing doses of radiation capable of causing mutations. Many laymen are becoming aware of this problem, although few yet seem to appreciate the quantitative aspects of it. Since there seems to be no minimum dose below which mutations will not occur, all unnecessary exposure to radiation should be avoided. Nevertheless, a glance at Fig. 19.8 will show that the problems of the medical use of x-rays (chest x-rays, dental x-rays, wart therapy, etc.) perhaps need more urgent attention than those raised by radioactive fallout. Unnecessary exposure to radiation, as with x-ray shoe-fitting machines, seems indefensible.

Human exposure to mutagenic radiation

Source	Dose (mrem/yr)
Natural background	102
Fallout	4
Nuclear power industry	0.003
Medical uses	73
Other	3
Total	182

Fig. 19.8 Estimated yearly radiation dosage to the gonads.

Third, the biologist sees mutations as an extremely valuable tool for discovering more about genetics. The discovery of sex linkage, discussed in the last chapter, is just one example of how mutant genes make clearer the mechanism of heredity.

Mutant genes have also been of great importance in providing clues to the way in which genes act to produce a given phenotype. Red eyes, nonclotting blood, waxy endosperm, etc., are not made up of DNA. They are simply the expression or product of gene activity. A discussion of genetics would not be complete without considering the steps by which a given genotype produces a given phenotype.

19.4 THE ONE GENE–ONE ENZYME THEORY

The most widely accepted theory on the method of action of genes grew out of the work of the geneticists G. W. Beadle and E. L. Tatum with the red bread mold *Neurospora crassa*. *Neurospora* is particularly well suited for genetic studies. Its life cycle is shown in Fig. 19.9. Note that the only diploid stage in the organism is the zygote, and it quickly undergoes meiosis. The vegetative stage of the organism is haploid. Thus each gene is present only once and the geneticist does not have to worry about the effect of recessive genes being masked by dominant ones. Another great

The life cycle of Neurospora

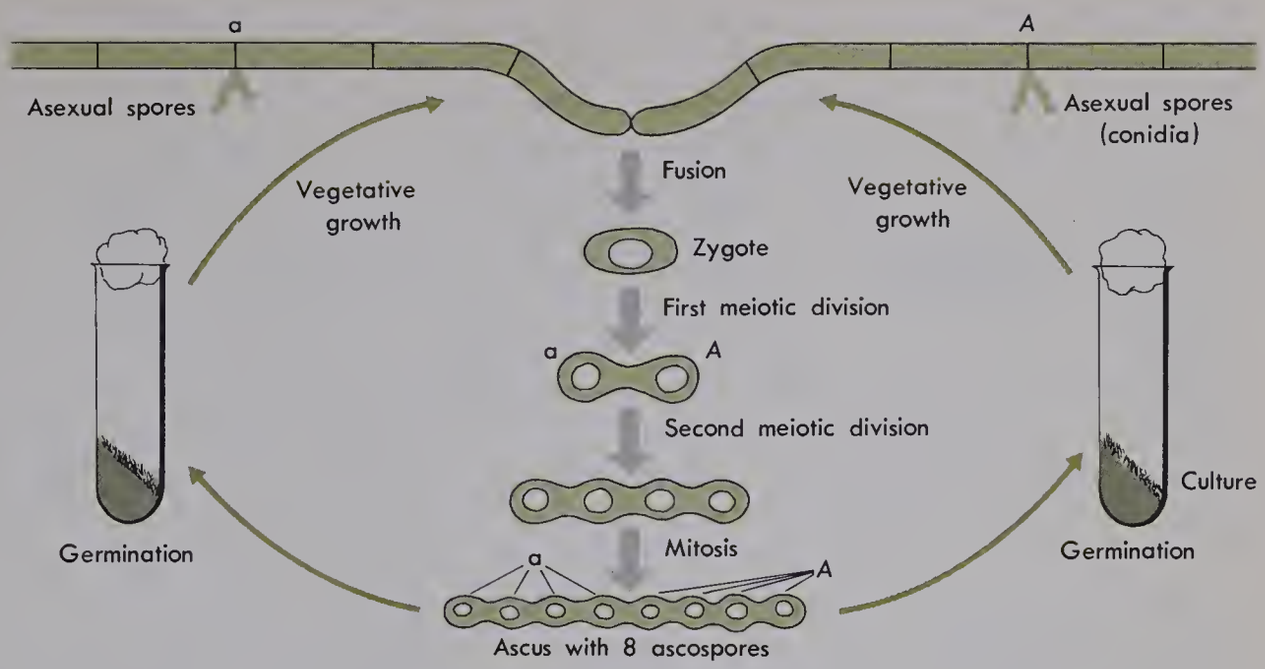


Fig. 19.9 Life cycle of *Neurospora*. The distribution shown here of the *A* and *a* alleles would occur only if no exchange of this locus took place during crossing over in the first meiotic division.

virtue is that the meiotic divisions of the zygote nucleus occur in an elongated structure, the ascus. The ascus is so narrow that the eight nuclei produced by the first and second meiotic divisions followed by one mitotic division are not able to slip past one another. Consequently, if the original zygote nucleus is heterozygous for some pair of alleles, and no crossing over of that locus occurs, the alleles will segregate at first meiosis. After the next two divisions, the ascus will have four spores at one end containing one allele and four spores at the other end containing the other allele. (What patterns might occur if crossing over of the loci *should* occur in the first meiotic division?)

This segregation of alleles in the spores of the ascus silences one criticism sometimes made of genetic studies, namely, that their analysis is always statistical. Sperm, pollen, or eggs display Mendelian ratios only when large samples are considered, because the products of one meiotic division become mixed at random with the products of other meiotic divisions. On the other hand, in *Neurospora* the nuclei resulting from a given meiosis are held in a fixed position and show the Mendelian ratios directly and exactly.

The experiments of Beadle and Tatum

Neurospora can be grown in test tubes containing a very simple ("minimal") culture medium. Sucrose, a few salts, and one vitamin, biotin, provide all the nutritional requirements for *Neurospora* to live, grow, and reproduce. From these relatively few and simple substances, it is capable of synthesizing all the many complex substances, such as proteins and nucleic acids, necessary for life. Beadle and Tatum exposed some of the asexual spores of one mating type of *Neurospora* to ultraviolet rays. These

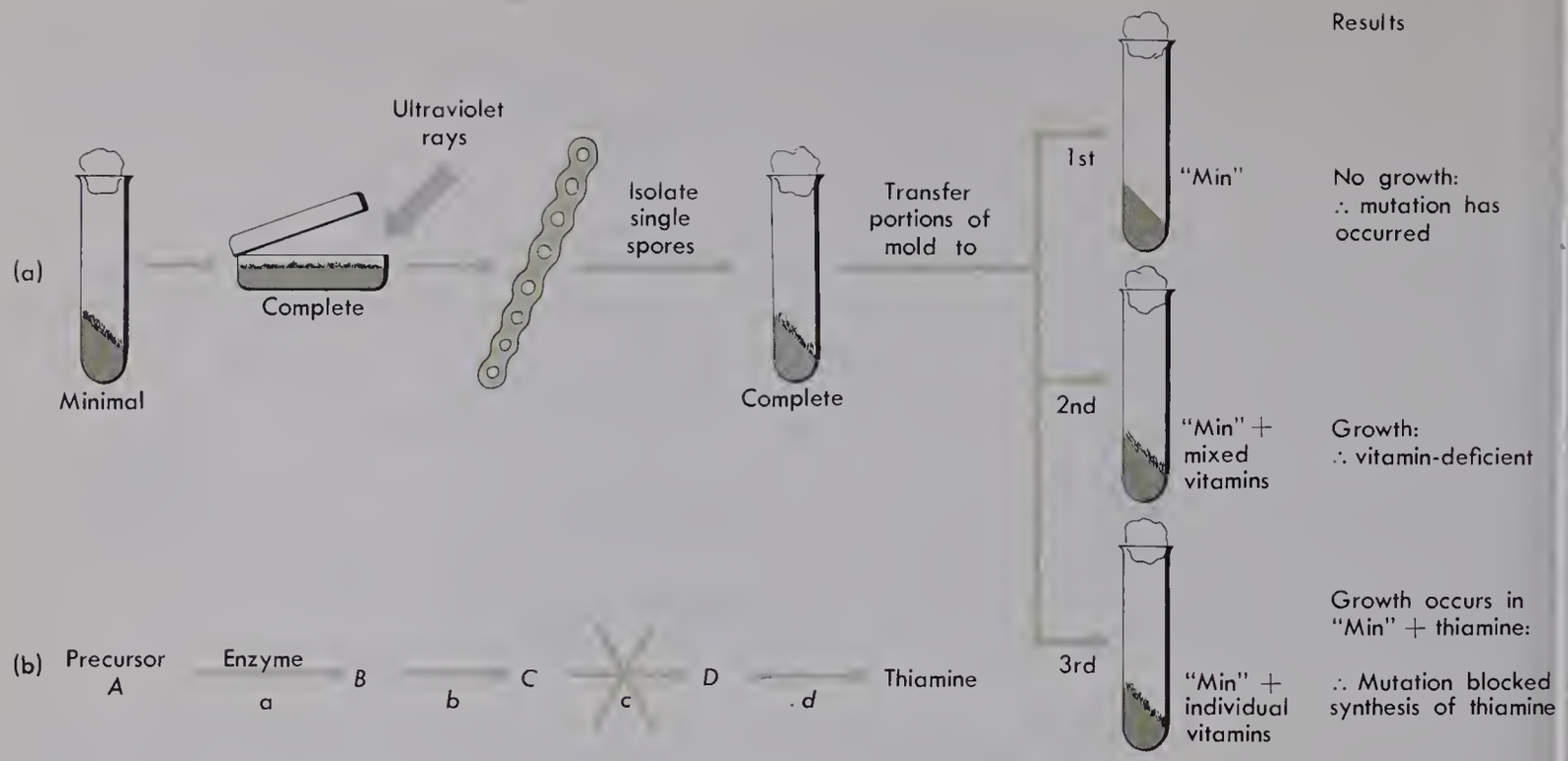


Fig. 19.10 Beadle and Tatum's (a) experiment and (b) hypothesis. Crossing the mutant strain with a normal strain showed that the ability to convert thiamine precursor *C* into precursor *D* was controlled by a single gene. Presumably the mutant gene could not produce the necessary enzyme.

spores were then allowed to germinate on a "complete" medium, that is, one enriched with various vitamins and amino acids. Once a vigorous growth had developed, it was allowed to mate with the other mating type. The ascospores produced as a result were then dissected out individually and each one placed on an additional quantity of complete medium. After growth had occurred, portions of each culture were placed on a minimal medium. Sometimes growth continued; sometimes it didn't. When it did not, the particular strain was then supplied with various vitamins, amino acids, etc., until growth did occur. Eventually, each deficient strain was found to be capable of growing on a minimal medium to which *one* accessory substance, for example, the vitamin thiamine, had been added. Beadle and Tatum reasoned that ultraviolet irradiation had caused a gene that permits the synthesis of thiamine to mutate to an allele that does not.

The manufacture of thiamine from the simple substances present in the minimal medium does not take place in a single chemical reaction but in a whole series of them. Like all chemical reactions in living things, each one requires the presence of a specific enzyme. By adding intermediate compounds (precursors) to the medium in which their mold was growing, Beadle and Tatum were able to locate just which step in the synthesis of thiamine was blocked in their mutant strain (Fig. 19.10). If they added to

the minimal medium any precursor further along in the process, growth occurred. Any precursor before the blocked reaction did not permit growth. They reasoned that the change of precursor C to precursor D was blocked because of the absence of the specific enzyme required. On this basis they created the "one gene–one enzyme" theory of gene action: each gene in an organism controls the production of a specific enzyme. It is these enzymes which then carry out all the metabolic activities of the organism, resulting in the development of a characteristic structure and physiology, the phenotype of the organism.

One might argue that Beadle and Tatum's experiment does not prove that only a single gene was involved. When the thiamine-deficient mutant is mated with a normal strain, though, the resulting asci contain four mutant spores and four nonmutant spores. Would this 50:50 ratio have occurred if more than one gene locus were involved?

The discoveries of Beadle and Tatum (for which they shared a Nobel Prize) shed new light on some interesting human diseases which were known to be hereditary. One of these, alcaptonuria, is a rather rare ailment in which the chief symptom is that the urine of the patient turns black upon exposure to air. Biochemical studies have shown that the disease results when the enzyme that catalyzes the conversion of homogentisic acid to maleylacetoacetic acid is lacking in the individual (Fig. 19.11). These substances are intermediates in the breakdown of the amino acid phenylalanine into compounds that can be oxidized in the citric acid cycle. When Step 4 is blocked, homogentisic acid accumulates in the blood. The kidney then excretes this excess in the urine. Oxidation of homogentisic acid by the air turns the urine black.

Alcaptonuria

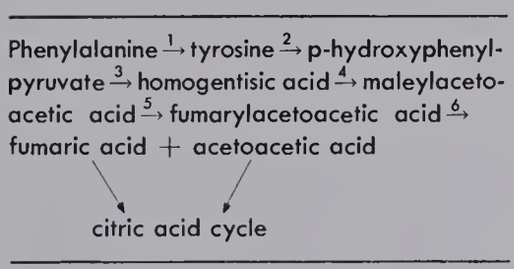


Fig. 19.11 Pathway of phenylalanine metabolism in humans.

PKU

Another hereditary disease, phenylketonuria (PKU), is caused by a blockage of Step 1. In this case, phenylalanine itself accumulates in the blood. The chief symptoms are a serious stunting of intelligence (most sufferers have to be confined to mental institutions), pale skin, and a tendency to epileptic seizures. The pale skin may result from a lack of tyrosine from which the pigment melanin (responsible for suntan and freckles) is formed. Both alcaptonuria and phenylketonuria are recessive traits. An individual must be homozygous for the mutant allele to show the trait. This is quite consistent with the one gene–one enzyme theory: so long as one nonmutant gene is present, the necessary enzyme is manufactured.

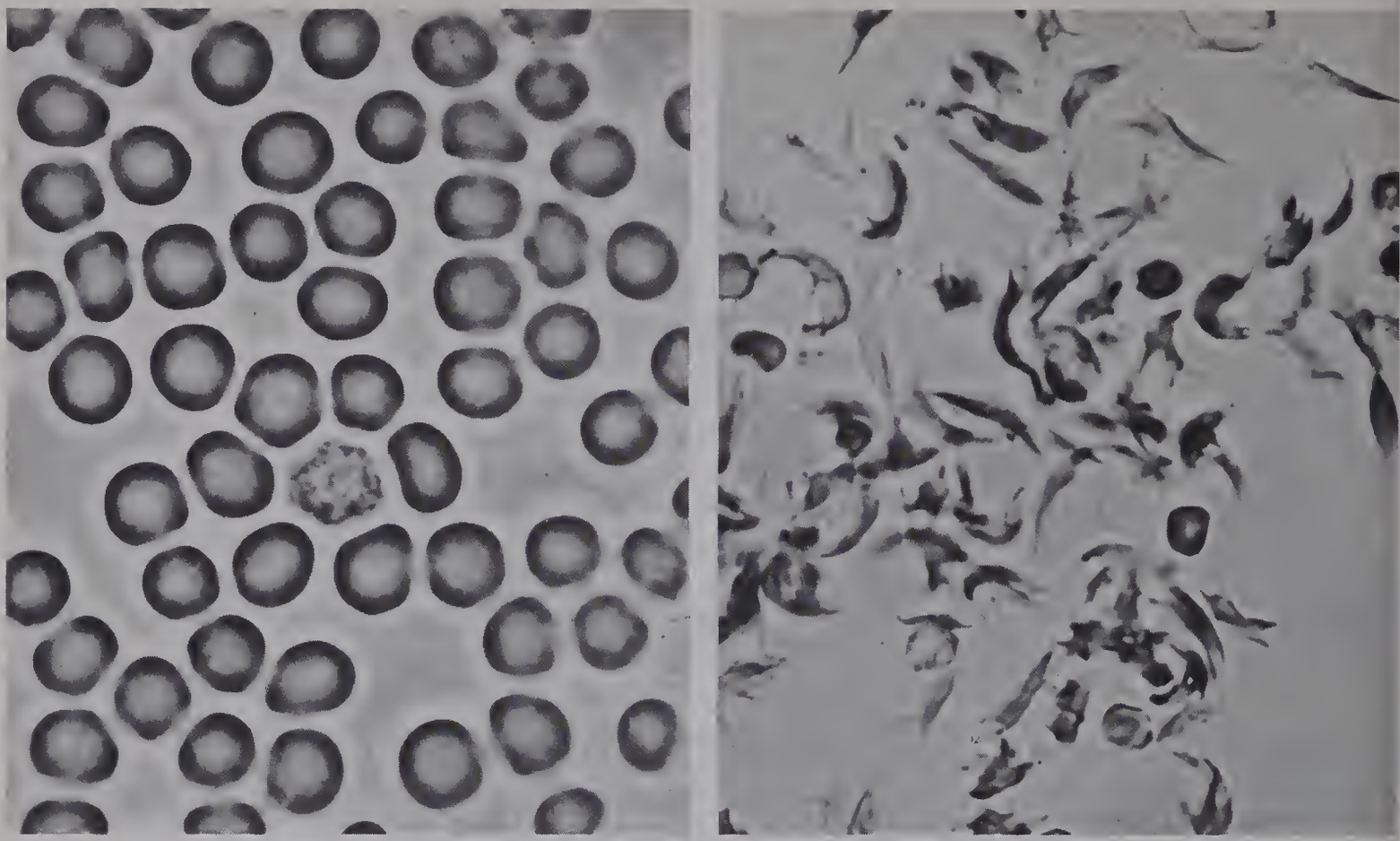


Fig. 19.12 Red blood corpuscles of a victim of sickle-cell anemia. Left: oxygenated. Right: deoxygenated. The shape of the cells when deoxygenated causes them to break easily. (Courtesy of Dr. Anthony C. Allison.)

Sickle-cell anemia

Perhaps the most thoroughly analyzed hereditary diseases are those in which abnormal hemoglobin molecules are produced. One of these is sickle-cell anemia. In individuals homozygous for the trait, the RBC's become crescent or sickle-shaped, particularly when passing through the capillaries (Fig. 19.12). This reduces the life span of the RBC's and a fatal anemia can result. Curiously, there is evidence that the mutant allele, when present in the single dose, confers on children some resistance to one kind of malaria. The allele is particularly prevalent among Negroes living in malarial regions. Studies of individuals having the allele show that they also have abnormal hemoglobin molecules in their RBC's. These are designated as Hb^S. Heterozygous individuals, with one normal allele and one mutant allele, have both normal hemoglobin (Hb^A) and Hb^S.

Structure of the hemoglobin molecule

Hemoglobin molecules are proteins consisting of four polypeptide chains (two "alpha" chains and two "beta" chains), each with the prosthetic group, heme. Each of the polypeptide chains is made up of about 150 amino acids. Chemical analysis of Hb^A and Hb^S reveals that they have the same sequence of amino acids in their alpha chains. Their beta chains are also identical except that at one place in the chain, the amino acid valine is substituted for the amino acid glutamic acid normally found at that point (Fig. 19.13).

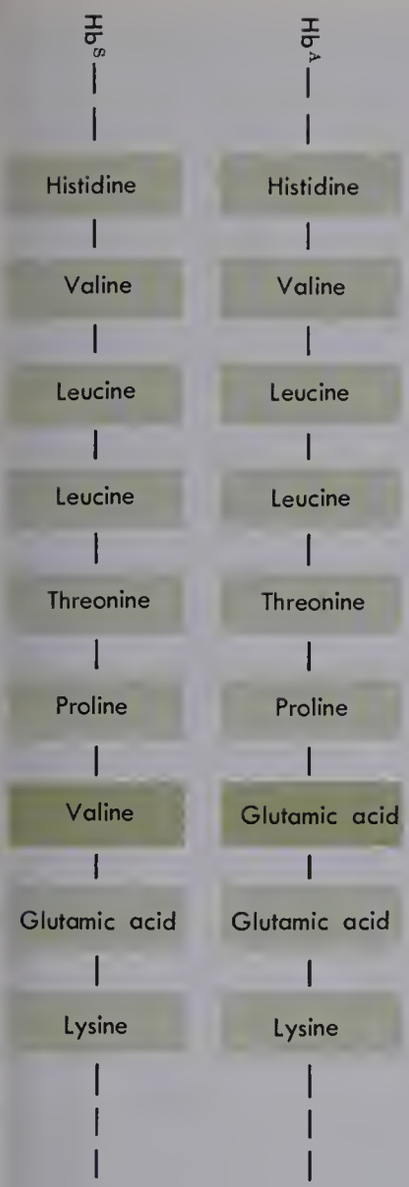


Fig. 19.13 Corresponding portions of the "beta" polypeptide chains of normal hemoglobin (Hb^A) and sickle-cell hemoglobin (Hb^S).

Other abnormal hemoglobins are known where alterations occur in the alpha chain of the molecule. These are produced by genes at a different gene locus from that of the sickle-cell allele. On this basis, the one gene–one enzyme theory can be refined into a one gene–one polypeptide theory. The evidence from sickle-cell anemia is that a gene not only controls the synthesis of a polypeptide but that its mutant allele may produce a polypeptide in which only one amino acid in the sequence has been altered.

19.5 PROTEIN SYNTHESIS

Most of the DNA of a cell is located in the nucleus. Most of the protein synthesis in the cell goes on in the cytoplasm. How, then, do the genes control the synthesis of protein in such a precise way? Biologists, chemists, and physicists in laboratories throughout the world are cooperating in an attempt to answer this question.

Protein synthesis occurs at tiny (150 A) cytoplasmic particles called **ribosomes** (Fig. 19.14). These are made up of three different molecules of **ribosomal RNA** with which are associated a number of different protein molecules. The RNA molecules are synthesized in the nucleolus. There do not appear to be specific ribosomes for the manufacture of specific proteins. In fact, the ribosomes from organisms as diverse as yeast and the rabbit seem interchangeable. While they are essential to protein synthesis, we must look elsewhere for the key by which the nature of the protein being synthesized is determined.

This substance is another kind of RNA called **messenger RNA.** Molecules of messenger RNA are synthesized within the nucleus and then pass out into the cytoplasm. They transmit the hereditary message from the DNA within the nucleus to the ribosomes out in the cytoplasm. Thus the name messenger RNA is well chosen.

DNA guides the synthesis of messenger RNA (and ribosomal RNA—see Fig. 19.15) in the same way that it guides its own duplication (see Section 19.2). A single strand of DNA serves as a template for assembling *ribo*nucleotides from the surrounding medium into a strand of RNA whose sequence of bases complements exactly the sequence of bases in the DNA molecules. The ribonucleotides are linked together with the aid of an enzyme called RNA polymerase. For every C on the DNA molecule, a G is inserted into the complementary strand of messenger RNA. So, too, every G picks up a C-containing ribonucleotide and every T picks up an A-containing ribonucleotide. A's on the DNA strand code the insertion of a uracil (U)-containing ribonucleotide. (There is no thymine in RNA.) When the job is completed, the single strand of messenger RNA leaves the DNA as a faithful transcription of it.

This theory of messenger RNA formation has been nicely supported. When a synthetic DNA consisting solely of T-containing nucleotides ("poly-T") is supplied with all four ribonucleotides (A, U, C, and G), and RNA polymerase, a messenger RNA molecule is formed which contains adenine only in the strand (poly-A).



Fig. 19.14 Polysomes. They manufacture proteins according to the instructions coded in the genes. (Courtesy of Alexander Rich.)



Fig. 19.15 An electron micrograph revealing gene transcription in the developing egg cell of the spotted newt. The long filaments are DNA molecules coated with protein. The fibers extending in clusters from the main axes are molecules of RNA from which the cell's ribosomes will be constructed. Note how transcription begins at one end of each gene, with the RNA molecules getting longer as they proceed towards completion. Note also the large number (up to 100) of RNA molecules that are transcribed simultaneously from each gene. The portions of DNA bare of RNA appear to be genetically inactive. (Courtesy of O. L. Miller, Jr. and Barbara R. Beatty, Biology Division, Oak Ridge National Laboratory.)

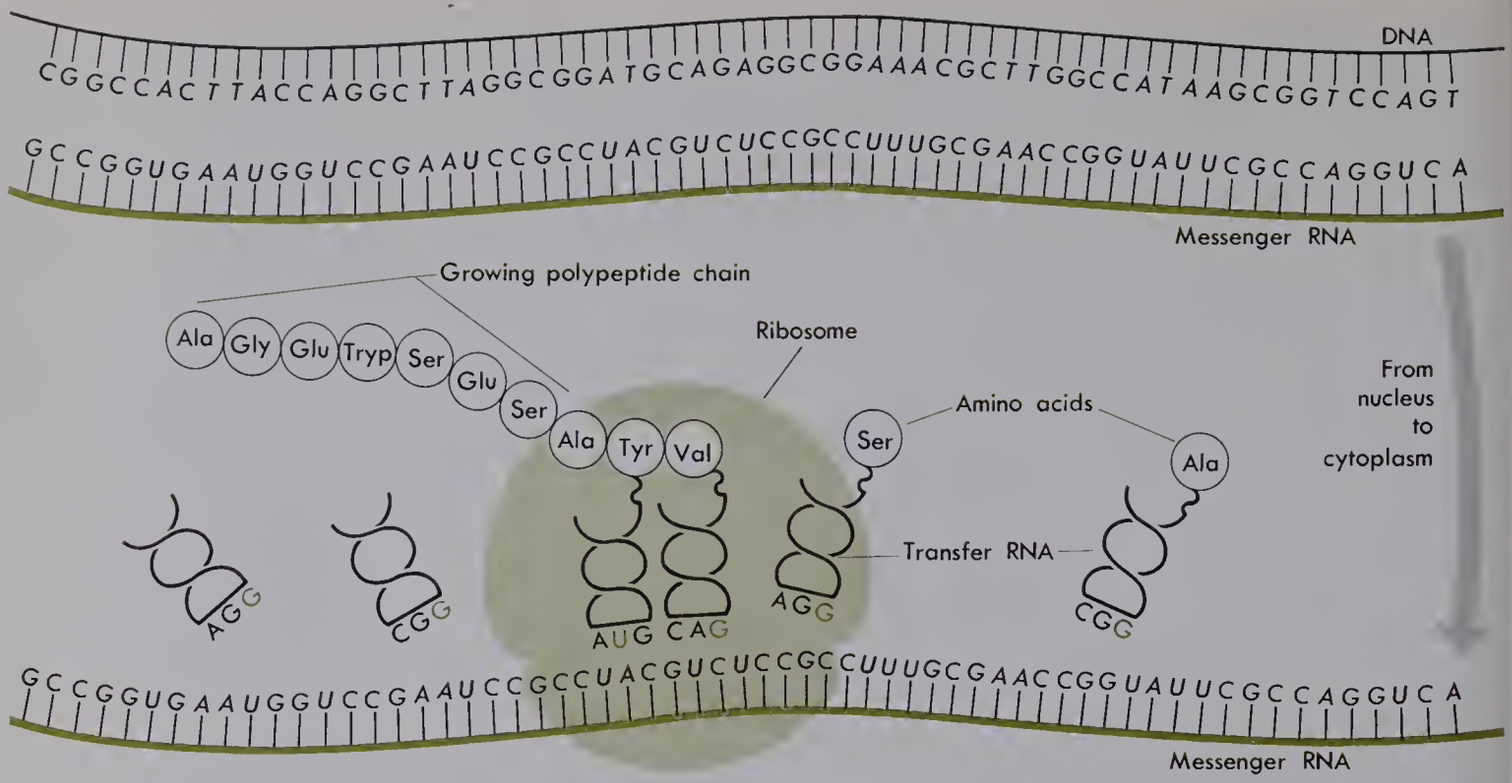


Fig. 19.16 Probable mechanism of the synthesis of a polypeptide according to the genetic instructions coded in DNA. The particular bases with which *transfer* RNA molecules "recognize" complementary codons on the *messenger* RNA molecules have been identified for the transfer RNA molecules shown. The use of colored letters for certain bases indicates that the actual base is a chemically modified form of the one indicated, but forms base pairs in the same way.

Messenger RNA can thus serve to transmit the hereditary message in the nucleus to the protein-making machinery of the cytoplasm. Several unanswered questions still remain, however. Proteins are made up of amino acids. How is the sequence of amino acids in a protein related to the sequence of exposed bases on a strand of messenger RNA?

Transfer RNA

This requires a third kind of RNA called **transfer RNA.** There are perhaps as many as 40–60 distinct kinds of transfer RNA molecules in the cell. Among these there is at least one specific transfer RNA molecule for each of the 20 amino acids used in protein synthesis. With the aid of a specific enzyme and ATP, each amino acid is activated and attached to a particular transfer RNA molecule.

The structure of several transfer RNA molecules has been determined. Each consists of a chain of from 75 to 85 ribonucleotides. Many of these are the normal RNA nucleotides (A, U, G, and C) and in places these bases link two portions of the chain in a double helix like the DNA molecule. Because of this, at least one loop is formed in the chain and, of course, it has two exposed ends (Fig. 19.16). The amino acid is attached to one of the free ends. It is thought that unpaired bases at a loop "recognize" complementary exposed bases on the messenger RNA molecule and unite with

them according to the usual rules of base-pairing. Twenty kinds of transfer RNA, each kind carrying its specific amino acid, could unite, theoretically, with those portions of the messenger RNA molecule whose bases were complementary to the exposed bases on the transfer RNA. This would bring the amino acids into sequence ready to be joined to form a polypeptide. Furthermore, this sequence would be controlled by the sequence of bases in the messenger RNA molecule and thus, ultimately, in the DNA molecule in the nucleus (Fig. 19.16).

Ribosomes

The union of transfer RNA and messenger RNA requires the presence of the ribosomes. Electron micrographs and biochemical analysis both suggest that the ribosomes attach to the end of a strand of messenger RNA and then move along its length, "reading" its sequence of bases. As they do this, they pick up the appropriate transfer RNA molecules, each with its amino acid. One at a time these amino acids become linked together to form the polypeptide chain. When the ribosome reaches the end of the messenger RNA strand, the polypeptide chain is complete. It and the ribosome are released. In this way, the message in the DNA molecule—its

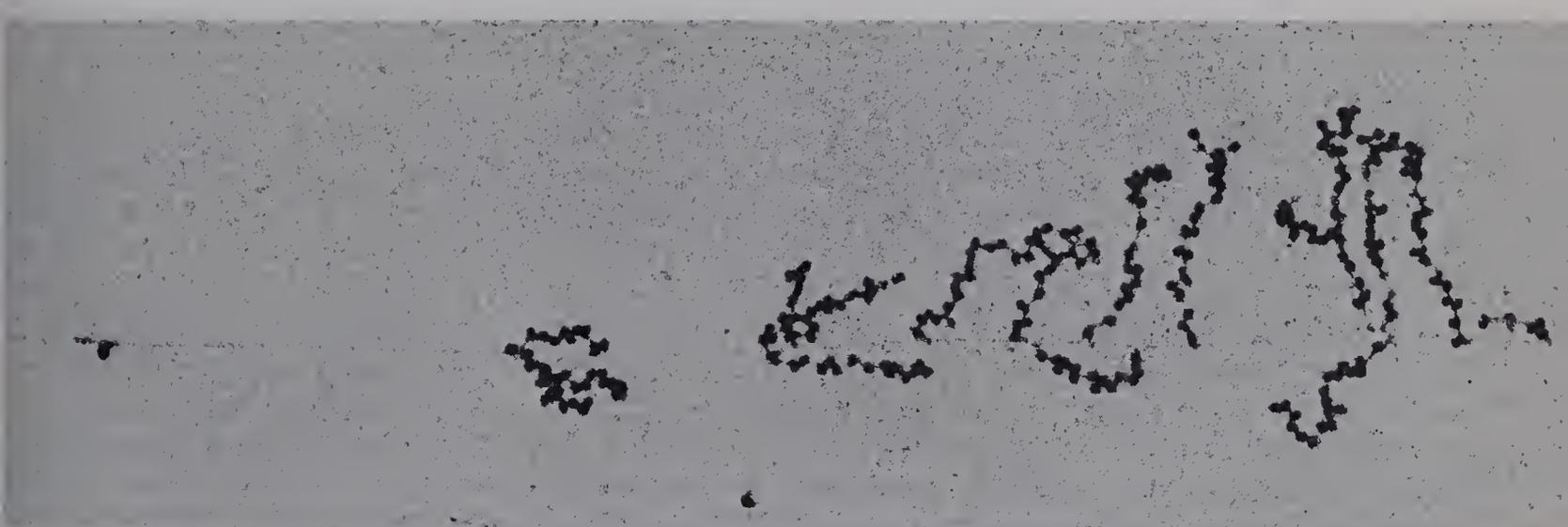


Fig. 19.17 Simultaneous transcription and translation of *E. coli* genes. The long (3 μm) fiber running from left to right is a segment of the *E. coli* chromosome. Extending from it are polysomes the size of which generally increases from left to right. Each polysome consists of a backbone of messenger RNA to which the ribosomes are attached. Each polysome is attached to the DNA fiber by a structure thought to be a molecule of RNA polymerase. Thus it appears that the DNA is *transcribed* by RNA polymerase molecules moving from left to right and the growing messenger RNA strands are *translated* by ribosomes moving along them in a proximal⟶distal direction. In *E. coli,* then, and perhaps in all prokaryotes, the transcription of DNA into messenger RNA and the translation of messenger RNA into polypeptide chains (not visible here) are closely coordinated. (Electron micrograph, 61,000 X, courtesy of O. L. Miller, Jr. from O. L. Miller, Jr., B. A. Hamkalo, and C. A. Thomas, Jr., *Science,* **169,** 392–395, July 24, 1970.)

DNA is transcribed *into RNA; RNA is* translated *into protein*

sequence of bases—which had been *transcribed* into a molecule of messenger RNA becomes *translated* into a specific sequence of amino acids in the polypeptide (Fig. 19.16).

Although a single ribosome can manufacture a polypeptide chain from a messenger RNA molecule, several ribosomes are usually engaged in the process at one time. One messenger RNA molecule with several ribosomes attached to it at various stages in assembling the polypeptide is called a polyribosome or **polysome.** These have been observed under the electron microscope (Fig. 19.17).

Here, then, is a mechanism by which a unique sequence of bases in the DNA of the chromosomes controls a unique sequence of amino acids in the proteins manufactured by the cell. The DNA of a cell may thus constitute the "master" copy of the hereditary information, while the messenger RNA is the "working" copy. An analogy may help to make this clear. In the recording industry, great care and expense go into the manufacture of a master negative of a record. It is far too valuable to be used in the molds that produce the thousands of wax impressions that will be sold to the public. Instead, working copies of the master are made from it. These copies, which are called "mothers," are then used in the molds to produce the final product.

19.6 THE CODE

A great deal of work has also been done in an effort to determine what sequence of bases in the messenger RNA molecule codes each of the amino acids. There are only four kinds of bases (A, U, C, and G) in the messenger RNA molecule, too few for each one to code a single amino acid. Pairs of bases could be arranged in 16 different ways (AA, UU, UA, etc.), but this is still short of the necessary number. Triplets of bases could be arranged in 64 different ways (Fig. 19.18) and thus would provide ample possibilities for coding 20 amino acids. In fact, we might expect two or three different triplets to code a single amino acid. Such a triplet code could also account for the presence of more than 20 different transfer RNA's in a cell with, in some cases, two or three different transfer RNA's each bringing the same amino acid to the growing polypeptide.

By manufacturing synthetic messenger RNA molecules and then supplying them with ribosomes, ATP, enzymes and all 20 amino acids, it has been possible to establish the triplets that code the various amino acids. For example, a synthetic messenger RNA that has only uracil (poly-U) produces a polypeptide containing the single amino acid phenylalanine. This suggests that the triplet UUU guides the incorporation of phenylalanine into the growing polypeptide chain. Similarly, synthetic poly-A messenger RNA guides the synthesis of a polypeptide containing only the amino acid lysine. Through the use of other synthetic messenger RNA molecules, it has been possible to correlate one or more triplets with each one of the 20 amino acids (Fig. 19.18). Because of this coding relationship, a triplet of bases is called a **codon.**

Three adjacent nucleotides comprise a codon

UUU, UUC — Phenylalanine (Phe)	CUU, CUC, CUA, CUG — Leucine (Leu)	AUU, AUC, AUA — Isoleucine (Ile)	GUU, GUC, GUA, GUG — Valine (Val)
UUA, UUG — Leucine (Leu)		AUG — Methionine (Met)	
UCU, UCC, UCA, UCG — Serine (Ser)	CCU, CCC, CCA, CCG — Proline (Pro)	ACU, ACC, ACA, ACG — Threonine (Thr)	GCU, GCC, GCA, GCG — Alanine (Ala)
UAU, UAC — Tyrosine (Tyr)	CAU, CAC — Histidine (His)	AAU, AAC — Asparagine (Asn)	GAU, GAC — Aspartic acid (Asp)
UAA, UAG — Chain terminators	CAA, CAG — Glutamine (Gln)	AAA, AAG — Lysine (Lys)	GAA, GAG — Glutamic acid (Glu)
UGU, UGC — Cysteine (Cys)	CGU, CGC, CGA, CGG — Arginine (Arg)	AGU, AGC — Serine (Ser); AGA, AGG — Arginine (Arg)	GGU, GGC, GGA, GGG — Glycine (Gly)
UGA — Chain terminator			
UGG — Tryptophan (Trp)			

Fig. 19.18 The genetic code. Although most of the codons have been assigned as a result of studies with *E. coli,* there is evidence that the code applies to all organisms. In most cases, several codons code for a single amino acid, and one codon may be "preferred" by one organism, another by a different organism.

"Stop" signals

Three of the 64 possible codons (UAA, UAG, and UGA) have not been found to code for any amino acid. In *E. coli* (and probably in all organisms) these three codons serve as punctuation marks. When the ribosome reaches them, the growth of the polypeptide chain is halted, and the chain is released ready to carry out its function in the cell.

The "start" signal

The codon AUG serves two related functions. When AUG occurs within the message being translated, it guides the incorporation of the amino acid methionine. However, all "messages" examined so far *begin* with AUG. It appears that AUG thus serves as a starting signal for the translation process. The transfer RNA molecule that binds to AUG at the start of a message is not the same as that which binds in the middle. (It does, however, carry a modified form of methionine which is usually removed from the polypeptide at a later time.) Just how the two AUG-recognizing transfer RNA molecules distinguish between AUG at the start of and AUG within a message is not yet known. Perhaps, as we shall see below, the presence of a short sequence of untranslated nucleotides just before the initiator AUG provides the distinguishing feature needed.

The nature of point mutations

To go back to our one gene–one polypeptide hypothesis, perhaps a mutant gene is simply a section of a DNA molecule in which one base pair has been altered. An alteration of a base pair in the DNA molecule would result in a corresponding alteration in the *messenger* RNA molecule. This, in turn, would provide a codon complementary to a different *transfer* RNA molecule. In some cases this shift might still code for the same amino acid. As you can see in Fig. 19.18, most of the amino acid codons can have the third base of the codon altered without changing the amino acid specified.

However, other base changes in the RNA would call for the insertion of a different amino acid into the polypeptide at that point. Such a change is a mutation.

A single base change accounts for the difference between Hb^A and Hb^S

The codons GAA and GAG code for glutamic acid (Fig. 19.18). In our example of Hb^A and Hb^S, the substitution of a single base, a U for an A in the middle position of the codon, would give a GUA or a GUG codon. Both of these have, indeed, been assigned to valine, the amino acid substitution in sickle-cell hemoglobin. Perhaps, then, the primary difference between a sickle-cell individual and a normal one is the presence of adenine (A) instead of thymine (T) at one spot in the part of a DNA moelcule that stores the information for the synthesis of the beta chain of hemoglobin. Although this change seems to be a minor one, the resulting substitution of valine for glutamic acid so alters the physical properties of hemoglobin that a fatal anemia is produced in the individual carrying both genes for the trait.

A single gene can mutate in many ways

Not all base changes result in an altered polypeptide; nevertheless, you can well appreciate that even in a "small" gene, such as that specifying the beta chain of hemoglobin, the number of possible mutations is very large. And, in fact, as the study of genes and gene action has progressed, it has become quite clear that a given gene may be mutated in a variety of different ways. In just a few years, almost 100 different mutations have been discovered in the two genes controlling alpha and beta chain synthesis in human hemoglobin. Each one of these results in a polypeptide with a single amino acid substitution somewhere along its length.

19.7 VERIFICATION OF THE CODE

The brilliant research which led to the codon assignments shown in Fig. 19.18 was carried out in test tubes. Perhaps for this reason and perhaps because the story this research told seemed almost fantastic, a number of biologists questioned whether such a system really operated in living organisms. What was needed to demonstrate this was to be able to determine the exact sequence of nucleotides in a gene (or a messenger RNA molecule) and see whether the sequence of bases corresponded to the sequence of amino acids in the protein coded by that gene (or synthesized by that mRNA molecule). Unfortunately, one cell contains, at most, two copies of each gene (with some exceptions). While a given gene may be transcribed into multiple copies of mRNA, most cells contain at any one time many kinds of mRNA, none of which is present in very large amounts. In order to determine sequences, a chemist needs plenty of purified material with which to work.

The problem of "sequencing" a gene

The life cycle of an RNA virus

A way out of this dilemma was presented by the single-stranded RNA bacteriophages. These viruses have a cycle of infection essentially like that shown in Fig. 19.3. Each virus particle consists of a protein coat inside which is a single molecule of single-stranded RNA rather than the DNA found in so many viruses (and shown in Fig. 19.3). This molecule of RNA has a dual function. It carries all the genes of the virus, of which three have

been identified. (Inasmuch as the whole molecule is only 3500 nucleotides long, it is unlikely that there are many more.) One of these RNA genes codes for an enzyme, an RNA polymerase, which can then use the entire RNA molecule as a template against which additional copies can be synthesized. This RNA ⟶ RNA copying of the genetic material is analogous to the DNA ⟶ DNA gene copying process we examined earlier in the chapter.

In providing the information from which the RNA polymerase can be *translated,* the RNA molecule of these bacteriophages is also serving the function of a messenger RNA. A second gene codes for the protein molecule out of which the virus coat is constructed. As is the case with DNA viruses, the machinery for translation (ribosomes, transfer RNA molecules, amino acids, and special protein factors) is that of the host cell, in this case *E. coli.*

The coat protein

The sequence of amino acids in the coat protein of several of these RNA bacteriophages has been determined. In each case, it has turned out to be constructed from a polypeptide of 129 amino acids. With this knowledge and the ability to grow vast numbers of virus particles from which the RNA can be extracted, it is now possible to attempt to verify the code. W. Fiers and his associates have recently succeeded in determining the precise order of A's, C's, G's, and U's in a stretch of RNA 608 nucleotides long extracted from the RNA bacteriophage MS2. A portion of their results is shown in Fig. 19.19.

The nucleotide sequence of MS2

A number of important findings quickly become apparent. The first place where the proposed initiator sequence AUG appears is at nucleotides 29, 30, and 31. Then, starting with the 32nd nucleotide and reading along in groups of three, we find that the amino acid predicted for each triplet by the codon table given in Fig. 19.18 establishes a sequence which is *exactly* the sequence which independent analysis has determined for the coat protein of the MS2 phage. Immediately following the 129th triplet appear the triplets UAA and UAG (Fig. 19.19). *Both* of these have been assigned the function of chain termination. (Why two terminators are needed is not known.) Then comes a sequence of nucleotides comprising 10 triplets for which no polypeptide product is known and which are thus probably not translated. Next is another initiator codon, AUG, followed by 19 nucleotides, the first 18 of which correspond to the first 6 amino acids in the RNA polymerase coded by the bacteriophage. What Fiers and his associates have done, then, is to reveal the coded information content of an entire gene—complete with start and stop signals—as well as portions of two other genes and the two intergenic "spacers" separating the three genes. In accomplishing this historic task, they have verified in the most direct way possible the validity of the genetic code and thus placed on an unshakable foundation one of the supreme achievements of the twentieth century.

Untranslated regions are found between genes

The code is probably universal

Is the code universal? Probably so. It appears that the same codons are assigned to the same amino acids and the same start and stop signals in such diverse organisms as *E. coli,* yeast, the tobacco plant, a species of toad,

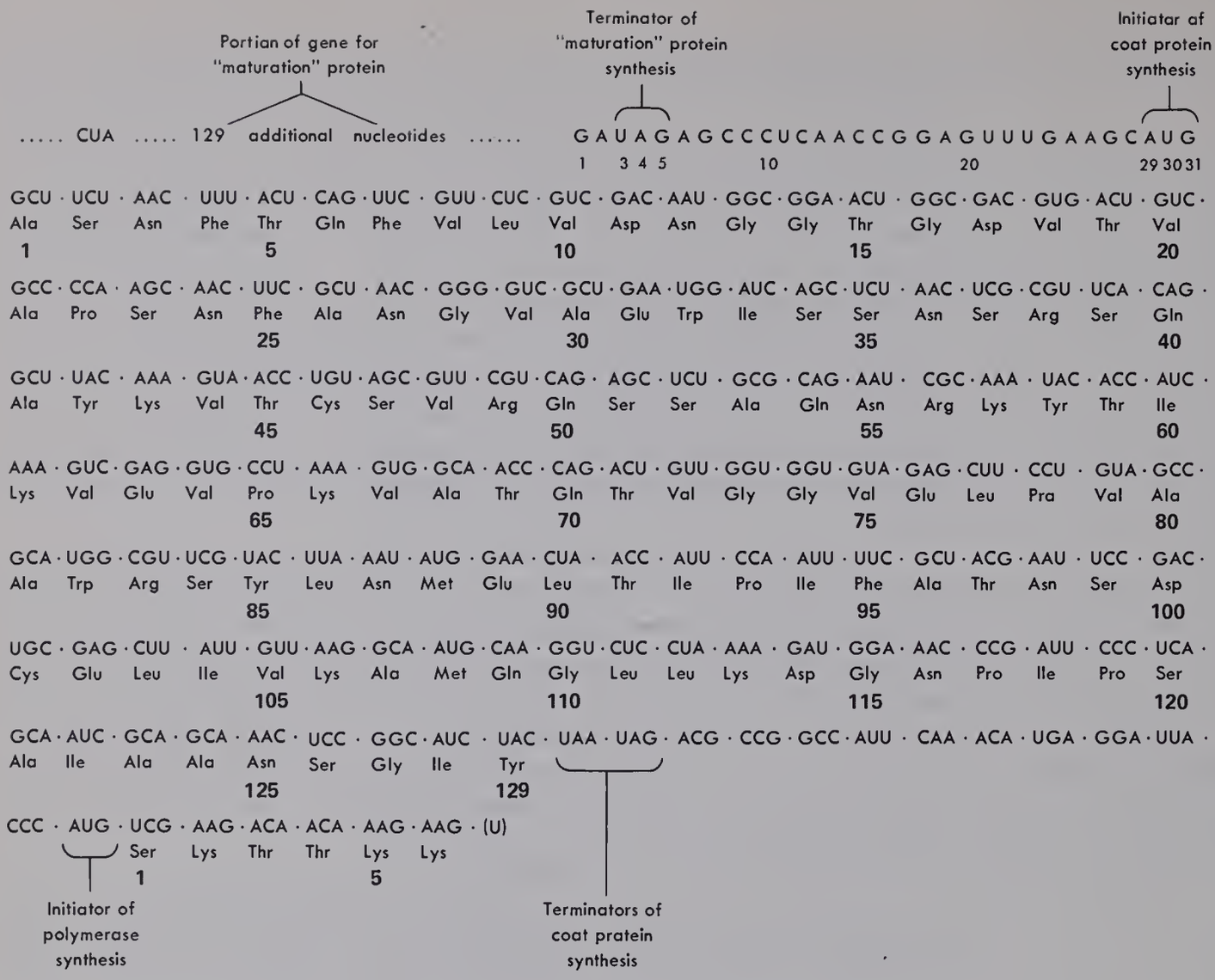


Fig. 19.19 Nucleotide sequence of a portion of the RNA of the MS2 bacteriophage. This sequence includes: (1) the entire gene for the coat protein of the virus (codons Numbers 1–129), (2) its initiation and termination signals, (3) portions of the preceding and succeeding genes, and (4) untranslated sections between the three genes. Analysis of the proteins synthesized by these genes has shown that the codon assignments given in Fig. 19.18 are, without exception, correct. Of the many synonymous codons, all but 12 are used in the coat protein gene. Analysis of the gene preceding it (only a portion of which is shown here) has revealed the remaining 12; therefore MS2 (like its host *E. coli*) uses every possible codon even though most are redundant. These data were obtained by W. Fiers and his associates.

the mouse, the rabbit, and the guinea pig. As you can see from Fig. 19.18, however, almost all the amino acids are coded by more than one codon. *Which* of the alternative codons for a particular amino acid is most often used may vary from species to species.

19.8 MULTIPLE ALLELES

A diploid cell contains only two of any particular set of multiple alleles

The mutant gene for Hb^S is an allele of the gene for Hb^A. All the other hemoglobin beta chain mutations are also allelic. We use the term **multiple alleles** whenever a variety of mutant forms of a single gene are known. Note that any one individual can have only two alleles (e.g. Hb^A, Hb^S), one on each chromosome. Within a population, however, many alleles may be

present. Over a dozen alleles have been found at a single locus in fruit flies that controls eye color.

Crossing over occurs within genes as well as between them

Using mutagenic agents, some 300 different mutations have been produced in a single gene on a bacteriophage chromosome. In fact, just as crossing over separates linked genes, so it can separate the parts of a gene. As we saw in Chapter 18 (Section 18.6), the closer two genes are on a chromosome, the less frequently they are separated by crossing over. We might guess from this that the separation of parts of a gene would be rarer still. This is so. It is so rare, in fact, that for any particular gene under study it is not detectable in an organism such as the fruit fly whose offspring number only in the hundreds. On the other hand, this phenomenon *can* be detected among the billions of offspring produced in a short period when bacteriophages infect a culture of their host cells. It has, in fact, been possible to map a linear sequence of intragene mutations for a single bacteriophage gene in the same way that the linear sequence of genes in a single fruit fly or corn chromosome is mapped. Probably any base in the chain of several hundred to several thousand needed to code a single polypeptide can be altered and, if it codes for a different amino acid, a mutation thus produced. We can feel confident that as the study of genetics progresses, multiple alleles will prove to be the rule rather than the exception.

If a dominant gene produces one polypeptide, does its recessive allele simply not make any polypeptide at all? Certainly this is not the case with the genes specifying abnormal hemoglobins. A person heterozygous for

Incomplete dominance

sickle-cell anemia produces *both* Hb^S and Hb^A in the same red blood cells. Such persons can be distinguished from those homozygous for Hb^A as well as from those homozygous for Hb^S. Many other cases are known where hybrids produce a phenotype distinguishable from that of either homozygous parent. This situation is called **incomplete dominance.**

19.9 REVERSE TRANSCRIPTASE

RNA tumor viruses

Among the RNA viruses are a number that infect the cells of mammals and birds, causing these cells to become malignant. That is to say, these so-called RNA tumor viruses cause cancers in a variety of experimental animals (and perhaps in man). A malignant or cancerous cell is one that divides repeatedly and uncontrollably giving rise to a tumor. The malignant characteristics of the first cell pass on to all the progeny cells in the same way as any other inherited trait.

The genetic material of cells is DNA but the genetic material of the RNA tumor viruses is RNA. How can infection by these viruses lead to the acquisition of a stable inherited trait in the recipient cell? In an attempt

Reverse transcriptase synthesizes DNA that is complementary to an RNA template

to answer this question, Howard Temin looked for the presence of an enzyme in infected cells that would be able to synthesize DNA complementary to the RNA of the infecting virus. He soon found it. Its discovery demonstrated that genetic information could flow from RNA to DNA as

well as in the way which we have been studying, i.e. DNA⟶RNA⟶ protein. This enzyme was named **reverse transcriptase.**

Are cancer-causing genes inherited?

The discovery of reverse transcriptase also provided a plausible explanation of the well-documented fact that many experimental animals *inherit* the ability to develop tumors. It is as though among the genes they inherit in the sperm and egg of their parents is one or more which someday will (1) initiate formation of a cancer and (2) initiate for the first time the production of RNA tumor viruses. Reverse transcriptase provides a mechanism by which the genetic information for both these functions could have been transcribed into DNA, which could then be incorporated with the rest of the genes of the animals' ancestors.

Reverse transcriptase as an experimental tool

The discovery of reverse transcriptase also provides a tool for making specific genes to order in the test tube. Provided one can secure adequate amounts of purified mRNA (for synthesis of hemoglobin A, for example), such a messenger could then serve as a template for the synthesis—catalyzed by reverse transcriptase—of the *gene*(*s*) for hemoglobin. In fact, several research groups have reported success in synthesizing DNA complementary to hemoglobin messenger RNA. Could such a gene be given to a victim of, say, sickle-cell anemia to enable him to manufacture Hb^A in addition to Hb^S and thus to acquire the much more desirable phenotype of a heterozygote? Such a possibility is only one of a number that raise the prospect of intervening in human genetics, or, as it is sometimes called, genetic engineering.

19.10 THE PROSPECTS OF GENETIC ENGINEERING

Experimental work of recent years clearly raises the possibility of genetic engineering in humans. A number of possible approaches are being explored. One involves isolating, or synthesizing, a specific gene and then finding a way for cells lacking this gene to acquire it, incorporate it into their own genotype, and express it.

The in vitro *synthesis of a gene*

The existence of reverse transcriptase provides one mechanism by which a single gene could be synthesized in the test tube, provided that adequate amounts of pure mRNA of that gene were available. Other methods may also be productive. In 1970, H. G. Khorana and his coworkers reported the successful synthesis of a gene coding for a tRNA molecule. While this gene was small (77 nucleotides) as genes go, the techniques used will surely be applicable to the synthesis of much larger segments of genetic information.

Isolation of a gene

Using extraordinarily clever techniques, Beckwith and his associates succeeded in 1969 in actually isolating a single bacterial gene (and parts of two others). This was the gene for β-galactosidase, an enzyme that hydrolyzes milk sugar, lactose, into its subunits glucose and galactose. These workers were able to prepare substantial quantities of this purified genetic information (consisting of some 4100 base pairs). Electron microscopy revealed molecules about 1.4 μm long (Fig. 19.20).

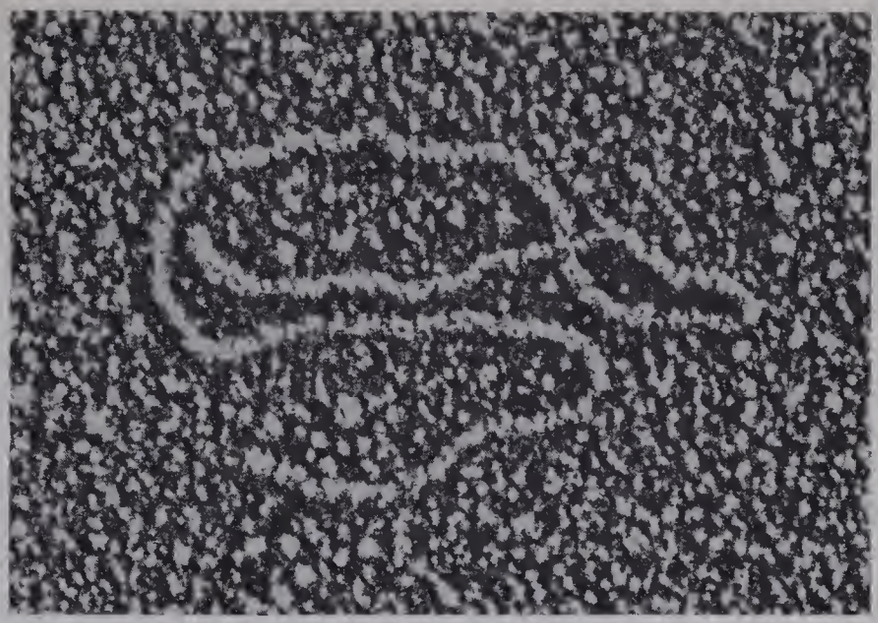


Fig. 19.20 Electron micrograph of a gene (172,000 X). This piece of DNA contains the gene that *E. coli* uses for the synthesis of the enzyme β-galactosidase. (Courtesy of Lorne A. MacHattie.)

The availability of purified genes brings up the problem of getting them into cells. Cells can pick up DNA preparations by endocytosis, but one would expect the vacuoles formed to fuse with lysosomes and the DNA to be degraded. Nevertheless, some success has been reported.

Human cells growing in vitro *acquire a new gene*

Workers at the National Institutes of Health reported in 1971 that they had succeeded in infecting human cells (growing in tissue culture) with bacteriophage DNA that included a gene involved in the metabolism of galactose. Prior to infection, the human cells lacked this enzyme, but after infection a tiny fraction of them began synthesizing the enzyme. Furthermore, they seem to have passed this newly acquired ability on to their offspring, indicating that they had not only taken up the genetic information but were able to duplicate it in an orderly way before each mitosis. Thus a transformation entirely analogous to the epoch-making transformation that Avery and his coworkers accomplished with pneumococci (see Section 19.1) has now been accomplished with human cells.

Using viruses to insert genes into cells

There are a number of DNA viruses that infect mammalian cells and (like the RNA tumor viruses described in the last section) their genetic information can become incorporated with the host's genes, and duplicated along with them prior to each mitosis. If one could attach a needed gene to such a virus *and if* one could block the harmful potentialities of the virus (they ultimately lead to the formation of malignant tumors in their host), a splendid tool would be available for inserting specific genes into a stable association with the genes of the host cell. Recently a whole set of genes for galactose metabolism was successfully attached to such a virus. The resulting complex will provide a good test of the feasibility of this approach.

The virus which causes fever blisters in man (herpes simplex) also carries a gene for an enzyme called thymidine kinase. A preparation of this virus treated so as to destroy its harmful properties has successfully been used to introduce the thymidine kinase gene into mouse cells (growing in tissue culture) which normally lack the gene.

A case of accidental gene therapy?

The type of genetic manipulation that the discoveries discussed here suggest is possible may already have been carried out inadvertently. For many years investigators have studied a virus that causes warts in the skin of certain rabbits. No known harmful effects to humans have been attributed to this virus even though one investigator inoculated himself with it. However, it is now thought that this virus carries the genetic information for the enzyme arginase, which catalyzes the breakdown (in the liver) of arginine to urea and ornithine (look back at Fig. 15.8). Subsequent study of laboratory workers who had at some time handled the virus showed that fully one-third of them had lower levels of arginine than is normal. Deliberate inoculation of experimental animals with the virus also lowers their arginine levels, suggesting a higher rate of enzyme activity. A very rare and serious genetic defect in humans is the inability to make arginase. If injection of these victims with the virus (which was recently carried out in two cases) succeeds in restoring this missing function on an indefinite basis, the age of direct gene therapy will already be here.

Inserting genes by somatic cell hybridization

Another approach to the insertion of new genes into a cell is to exploit the technique of cell fusion discussed in the last chapter (see Section 18.8). Henry Harris and his colleagues at Oxford have succeeded in fusing embryonic chick cells with mouse cells. In so doing, some of the mouse cells acquired a chick gene for an enzyme that the mouse cells lacked, and they were able to duplicate this gene, pass it on to their progeny, and express it through the formation of the enzyme.

The various techniques described above show real promise of ameliorating human genetic defects that involve only a single gene locus, e.g. Hb^S and PKU. Unfortunately, many of the more common human ailments with a genetic basis (e.g. diabetes mellitus) probably involve several or many genes. Whether suitable techniques for dealing with these can be devised remains to be seen. Some possible approaches will be examined in Chapter 21. While some approaches will undoubtedly not be practical, the time when we will be able to manipulate human genetic material in ways that would have been considered impossible a decade or two ago seems to be just ahead, if, indeed, it is not already upon us. The potentialities which these tools possess raise serious ethical questions. Technological innovations, the varied uses to which they are put, and the overall impact of them on society have often arrived with little advance notice and opportunity for thorough evaluation. The vistas which recent developments in genetic engineering open up suggest that we would do well to begin now to come to grips with the ethical and legal issues involved rather than wait until the technology is fully developed.

19.11 THE ACTION OF THE TOTAL GENOTYPE

Mendel knew that an understanding of the rules of inheritance could come only from the study of simple, clear-cut traits. Much of the later work in genetics has necessarily followed this tradition. Most of the information discussed in this chapter has come from the study of simplified, and thus

more manageable, genetic systems (e.g. the RNA virus MS2). However, clear-cut traits, like hemoglobin type or the presence of a single functioning enzyme, represent only a small part of the story of how genes guide the development and functioning of an organism. Although genetics has made only a beginning at relating the total organism to its complete hereditary blueprint, three important features already stand out.

A single gene may have many effects

One is that a single gene may have many effects upon the organism. Although a single gene produces a single polypeptide (usually), the activity of that polypeptide (or the protein of which it is a part) in different parts of the organism may lead to different effects. Furthermore, effects produced in one part of the organism, as a direct result of gene action, may in turn upset natural balances in other parts of the organism. A whole series of functional and structural changes may thus follow indirectly from some primary gene action. The inheritance of the trait "frizzle" in chickens illustrates this. Chickens homozygous for the trait have defective feathers which break easily and provide little heat insulation for the bird. Presumably, the mutant alleles produce a protein which does not permit proper feather development. The poor insulating qualities of frizzle feathers lead to increased heat loss from the bird. This, in turn, triggers a whole sequence of homeostatic responses resulting in marked structural and functional changes (Fig. 19.21). The variety of symptoms found in many of man's hereditary diseases, for example the pale pigmentation and seriously lowered I.Q. in phenylketonurics, also illustrates this principle.

A single trait is influenced by many genes

A second rule is that the expression of any trait is influenced by a large number of genes. One gene may result in a pea flower being red rather than white. Many other genes are necessary to produce the structure and arrangement of the petals. Going still further, no traits for flower color could be expressed without a coordinated activity of many genes guiding the development of functional roots, stems, and leaves.

Even such a simple trait as the ability to metabolize a small molecule like phenylalanine (Fig. 19.11) requires the action of a number of enzymes. Each of these is constructed from one or more polypeptide chains which, in turn, are each synthesized by a specific gene. Studies in a number of microorganisms have shown that in some cases the genes responsible for the synthesis of *related* enzymes are located adjacent to one another on the chromosome. In *E. coli,* for example, the genes that produce the enzymes necessary for the organism to metabolize the disaccharide lactose are located adjacent to each other in one region of the organism's chromosome. At one end of this chain of genes is a special gene, the operator, which turns the others on and off as conditions within the cell dictate. The combination of an operator gene and the genes that it controls is called an **operon.** The close linkage of functionally related genes is surely of value to the organism. By grouping such genes in operons, the production of all the enzymes needed for a given metabolic activity can proceed simultaneously and in accurately coordinated amounts.

The operon

How many genes does it take to build an organism? No one knows exactly. One can measure the amount of DNA found in each cell of an

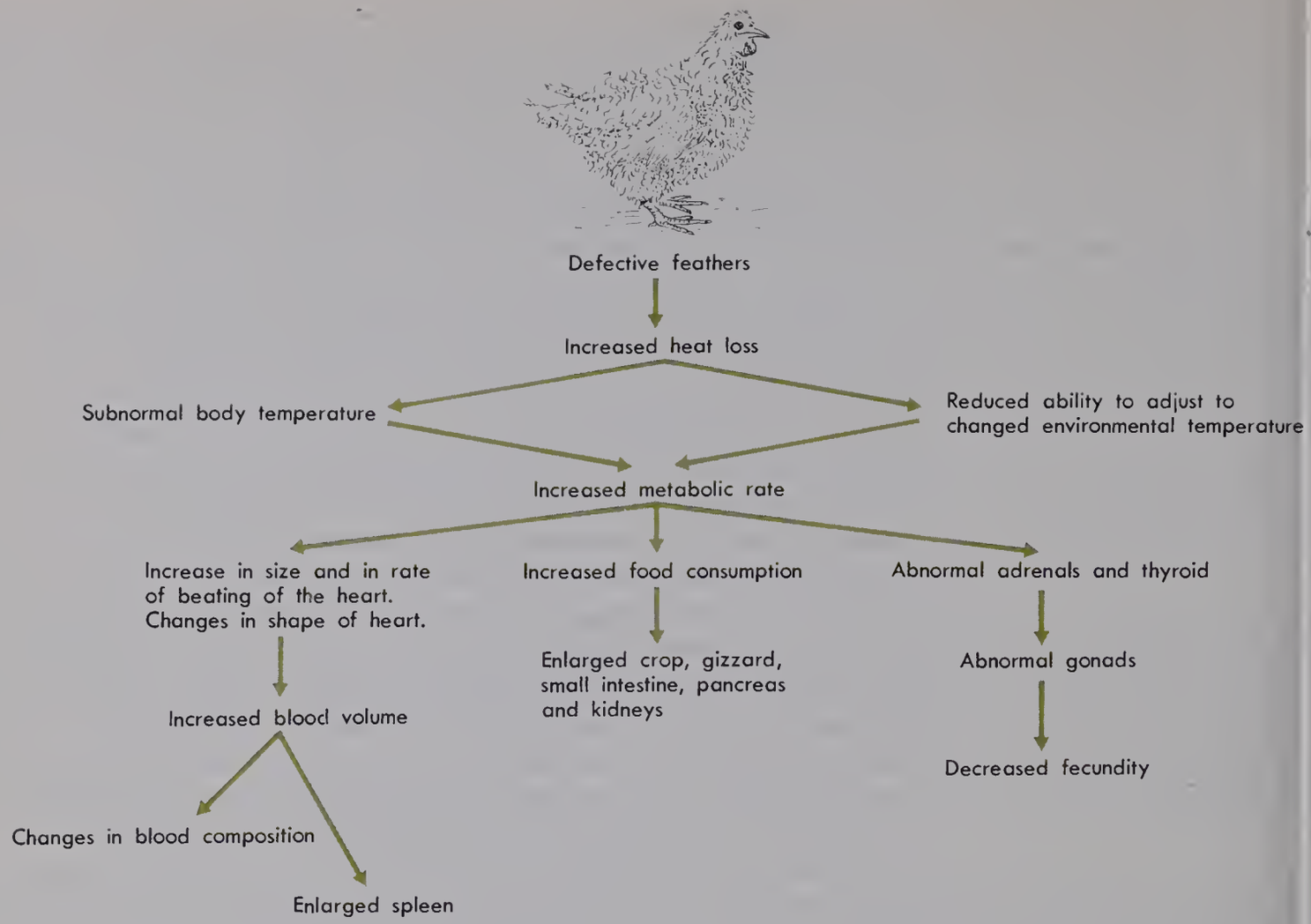


Fig. 19.21 Multiple effects of the gene for "frizzled" feathers in chickens. A whole series of secondary effects stem from the primary effect of the gene. (Adapted, with permission, from W. Landauer's "Temperature and Evolution," *Biological Symposia,* Vol. 6, The Jaques Cattell Press, Inc.)

Estimating the number of genes in a cell

organism (and it *generally* increases with the complexity of the organism) and from this determine the number of base pairs present. Since most polypeptides contain from 150 to 300 amino acids, we can assume that genes contain some 450–900 base pairs each. With this information you might proceed to estimate the number of genes present in each species. Such an estimate would, however, rest on the additional assumption that all the DNA in the cell is genetically active. It is now quite apparent that such is *not* the case. The cells of organisms other than bacteria contain varying amounts (20–60%) of *repetitive DNA,* that is, identical—or at least similar—sequences of DNA repeated over and over. Some of this repetitive DNA *is* genetically active but represents multiple copies of a *single* gene. Some of it does not appear to be genetically active, i.e. it is not transcribed or translated. This latter material is associated with the centromere region of the chromosome. Its function is still a matter of speculation. Because of the complication presented by repetitive DNA, estimates of the size of the genetic dictionary needed to build higher organisms are extremely uncer-

Repetitive DNA

tain. With *E. coli,* the situation is clearer. *E. coli* has no repetitive DNA and, except for the genes for rRNA, no multiple gene copies. Therefore, *E. coli* probably contains something of the order of 2000 to 3000 distinct genes. The values for *Drosophila* and man are surely much larger, but how much so remains to be determined.

E. coli *has 2-3,000 genes*

The action of genes is dependent also upon the environment. The ability to manufacture chlorophyll is under gene control, but plants cannot do the job unless they are exposed to light (look back at Fig. 9.5). If victims of the hereditary defect phenylketonuria (PKU) are identified very early, it is possible to prevent the mutant genes from expressing themselves. This is accomplished by reducing the amount of the amino acid, phenylalanine, in the diet of the patient.

Heredity and environment

Many farmers today raise hybrid corn varieties. The seeds, produced through extensive breeding programs, are of a very uniform and desirable genotype. The plants one farmer raises from the seeds may, however, vary greatly in phenotype from those raised by another farmer. Soil, temperature, water supply, and fertilizer are just a few of the many environmental influences that affect the ultimate phenotype.

Thus the genes an individual organism inherits determine the potentialities that may be achieved in the course of the entire growth and development of that organism. The environment establishes to what degree these potentialities will actually be achieved.

EXERCISES AND PROBLEMS

1. Distinguish between multiple factors and multiple alleles.
2. Neglecting crossing over, how many different kinds of ascospores can *Neurospora crassa* form by random assortment of its chromosomes? The haploid number is 7.
3. Besides DNA and RNA, what other important biological substances contain the purine adenine?
4. Is it correct to say that in the ascus produced by a heterozygous (Aa) *Neurospora* zygote, four spores will contain *A* and four spores will contain *a?* Explain.
5. Why can a definite prediction be made in Question 4 when you could not make a definite prediction in Question 4 at the end of Chapter 17?
6. What sequence of bases in messenger RNA will be coded by the following triplets in the DNA molecule: GCT, GAT, CCA, AAA, AGT?
7. Why are haploid organisms especially useful in genetics studies?
8. It is possible to buy sorghum seeds ¾ of which germinate into green seedlings and ¼ of which germinate into albino seedlings. The albino seedlings soon die. Why?
9. What was the genotype of the sorghum plants that produced the seeds in Question 8?
10. Outline all the steps which must be taken by a plant breeder in order to be sure that he is offering sorghum seeds that will demonstrate the 3:1 ratio in Question 8.

11. Why are organisms that reproduce rapidly and produce large numbers of offspring so useful in genetics studies?
12. It has been suggested that pocket watches should not have luminous dials but it is all right for wrist watches to have them. Why the difference?
13. Would Weismann have appreciated this difference?
14. Why are most mutations recessive?
15. What inorganic ions would you expect to add to sucrose and biotin to make a minimal medium for growing unmutated *Neurospora?* Why?
16. Minimal medium for *Neurospora* requires the presence of biotin while minimal medium for *E. coli* does not. Does this mean that biotin does not participate in the metabolism of *E. coli* cells? Explain.
17. Define a gene. Is this the same way you defined it at the end of the previous two chapters?
18. Show how asexual reproduction in *Bryophyllum* (see Fig. 16.4) depends on the duplication of (a) cells, (b) subcellular structures, (c) individual molecules.
19. Why are organisms (e.g. plants, *Neurospora, E. coli*) that can reproduce asexually so useful in genetics studies?
20. Using your knowledge of meiosis, predict what arrangements of *A* spores and *a* spores could occur in the ascus produced by a strain of *Neurospora* heterozygous for these alleles.
21. The first gene of the bacteriophage MS2 ends with a UAG termination signal, shown as nucleotides 3, 4, and 5 in Fig. 19.19. The next gene starts with AUG at positions 29, 30, and 31. Is the number of nucleotides in the intergenic region divisible by 3?
22. Some MS2 phages occasionally misread the UAG terminator (positions 3, 4, 5—see Fig. 19.19) and instead of stopping translation of the first gene, they insert the amino acid serine (Ser) at that point and *continue* with translation. Keeping in mind your answer to the previous question, what is the next possible "stop" signal?
23. Using the table of codon assignments (Fig. 19.18), give the sequence of amino acids that would occur in the aberrant, extra, portion of the protein synthesized as described in the previous question. (The existence of such a protein has been verified.)

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20 SEXUAL REPRODUCTION IN PLANTS

Alternation of Generations

The Problems to be Solved

Mosses

Ferns

Gymnosperms

Angiosperms

The Flower and its Pollination

The Seed

Seed Dispersal: the Fruit

Germination

20.1 ALTERNATION OF GENERATIONS

Sexual reproduction involves the two processes of fertilization and meiosis. In fertilization, the nuclei of two gametes fuse, raising the chromosome number from haploid to diploid. In meiosis, the chromosome number is reduced again from the diploid to the haploid condition. Whatever variation there may be in the details from one organism to another, these two activities must occur alternately if sexual reproduction is to continue.

Gametophyte and sporophyte generations

In plants, fertilization and meiosis divide the life of the organism into two distinct phases or "generations." The **gametophyte** generation begins with a spore produced by meiosis. The spore is haploid and all the cells derived from it are also haploid. Among the cells produced by this generation are the gametes. When two gametes fuse, the **sporophyte** generation begins. The sporophyte generation thus starts with a zygote. It contains the diploid number of chromosomes and all cells derived from it by mitosis are also diploid. Eventually though, certain cells will undergo meiosis, forming spores and starting the gametophyte generation once again.

20.2 THE PROBLEMS TO BE SOLVED

Plants probably evolved from aquatic ancestors, the green algae. Although plants can be found today growing in water, they are primarily associated with life on land. The members of each group of plants have a variety of morphological and physiological adaptations which enable them to live away from the aquatic environment. How plants solve the problem of securing water and keeping their cells moist was discussed in Part III. In this chapter, we shall examine the ways in which the plants have solved the problem of reproducing sexually on land.

The problem is really twofold. Gametes are single cells and quite delicate. In cross-fertilization, some mechanism must be present to enable the two gametes to reach each other safely. The problem is compounded in plants by their not having the power of locomotion. The inability of plants to move about also creates a problem of how to disperse the offspring produced by sexual reproduction to locations far enough away from the parent plant that they can receive the sunlight, water, and soil minerals they need. The twin problems of bringing the gametes together in a protected environment and dispersing the species to new locations have been solved in different ways by the various groups of plants.

Cross-fertilization and dispersal

20.3 MOSSES

A bed of moss consists of masses of leafy shoots which, being haploid, belong to the gametophyte generation (Fig. 20.1). In the common haircap moss, *Polytrichum commune,* the leafy shoots are of three kinds: female, male, and sterile. (The latter do not participate in sexual reproduction and need not concern us here.) The male shoots are easily distinguished from the other two by their flat top. A longitudinal section through the tip of a male plant reveals several of the male reproductory organs, the **anthe-**

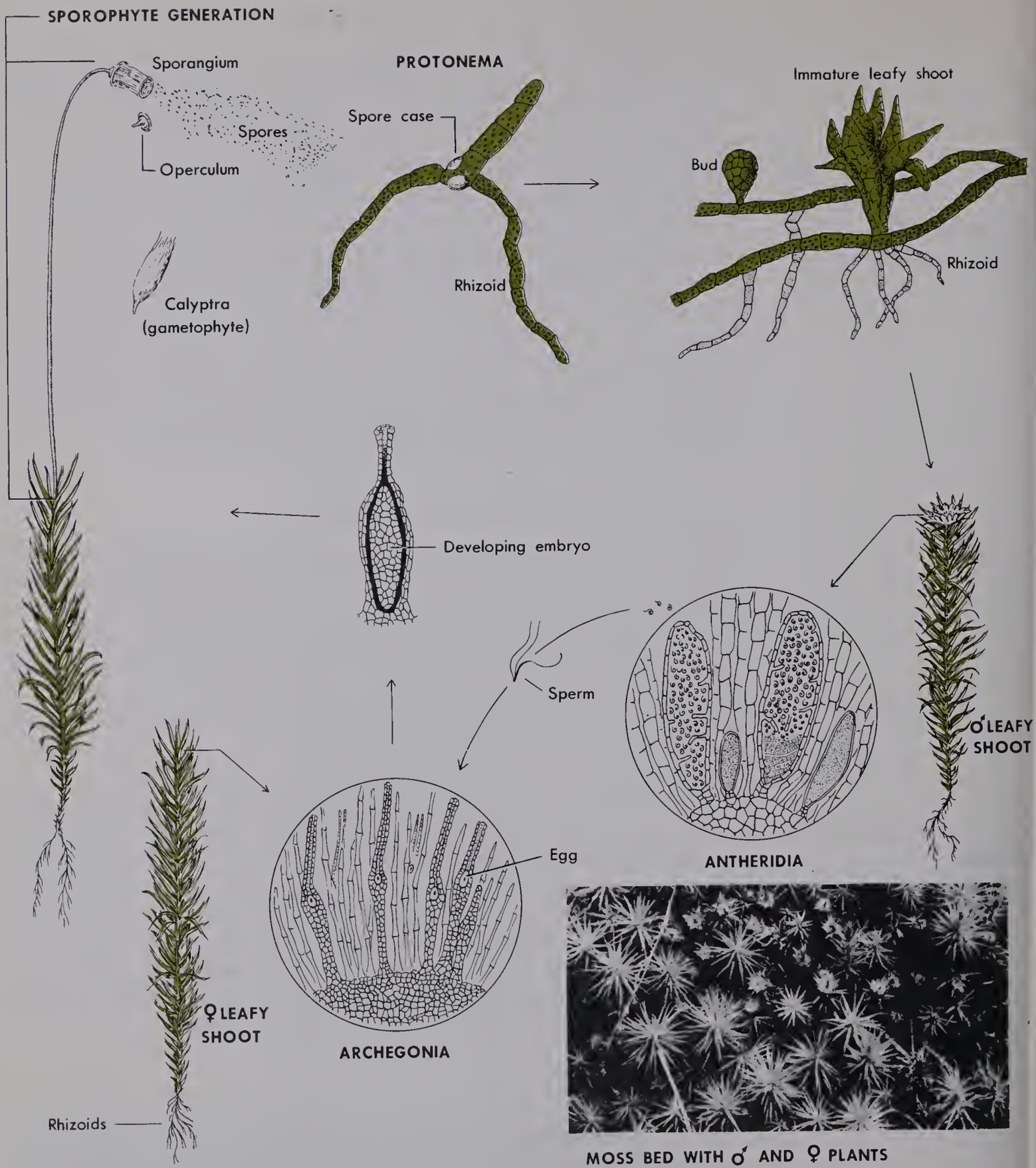


Fig. 20.1 Life cycle of the common haircap moss *Polytrichum commune.* In order for fertilization to take place, there must be surface water in which the sperm can pass from the male plant to the female plant.

ridia. Each is filled with sperm. A similar section through the female plant reveals several bottle-shaped **archegonia,** the female reproductory organs. Each of these contains a single egg in a chamber near its base. In early spring, if there is plenty of water present, the sperm are released from the antheridia. The splashing of raindrops distributes the sperm to nearby plants. On female plants, the sperm swim to the archegonium, probably following a concentration gradient of sucrose diffusing from the archegonium. The sperm swim down the canal to the egg, and fertilization takes place. The resulting zygote is the first cell of the sporophyte generation.

The gametophyte generation accomplishes sexual reproduction

Mitotic divisions of the zygote produce the mature sporophyte generation (Fig. 20.1). It consists of:

1. A foot, which absorbs water and minerals from the parent gametophyte. Although the cells of the sporophyte generation contain chlorophyll, some food materials may also be absorbed from the parent gametophyte.
2. A stalk, which grows a few inches up into the air.
3. A **sporangium,** which is formed at the tip of the stalk. Within it are located spore mother cells. The opening to the interior is sealed with a lid, the operculum. The entire sporangium is covered by a calyptra. The calyptra is derived from the wall of the old archegonium. (Thus it is actually a part of the gametophyte generation.) It is the calyptra that is responsible for the common name of this species.

During the summer, each spore mother cell in the sporangium undergoes meiosis, producing four spores, the start of the gametophyte generation. Late in the summer, the calyptra and operculum become detached from the sporangium. Lowered humidity causes the ring of teeth within the opening to the sporangium to bend outward. In so doing the spores are ejected. Their small size enables them to be transported great distances by the wind.

The sporophyte generation accomplishes dispersal

If a spore comes to rest in a suitable location (moist and shady), it will germinate to form a filament of green cells called a **protonema.** A resemblance between the protonema and certain filamentous green algae has suggested to botanists that the mosses evolved from algae. Soon buds appear on the protonema and from these buds develop the leafy shoots—male, female, and sterile—that started our story.

What are the roles played by these two stages in the moss life cycle? The gametophyte generation produces the gametes and is responsible for the carrying out of sexual reproduction. The process requires ample water, however, and in a dry spring sexual reproduction often fails to occur.

Even though it may manufacture some of its own food, the sporophyte generation is completely dependent upon the gametophyte for its water and minerals. Its contribution to the success of the species is the myriads of wind-blown spores which it produces. These disperse the species, enabling it to colonize new habitats.

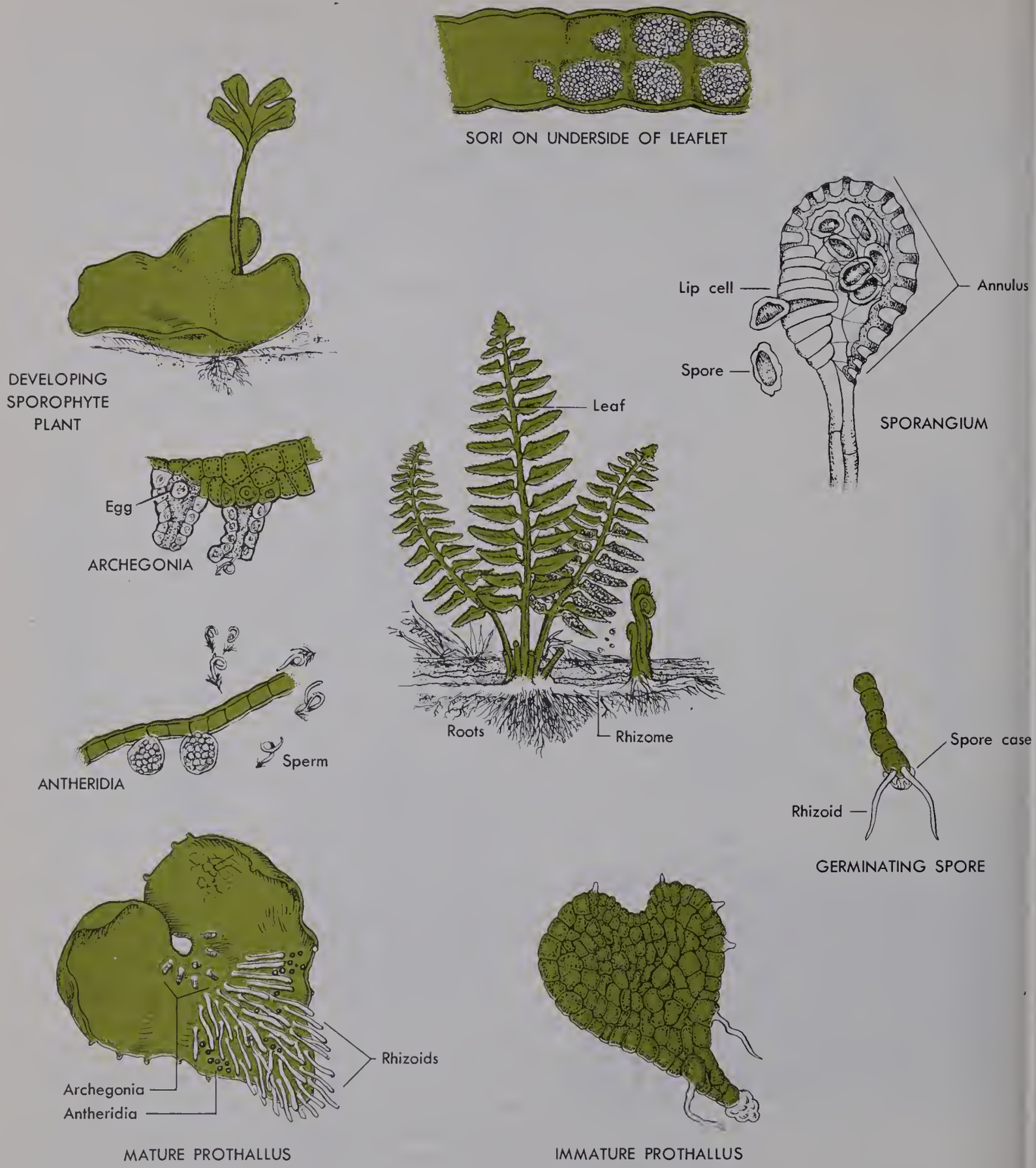


Fig. 20.2 Life cycle of a typical fern. Cross-fertilization is the rule; that is, the sperm swim from one prothallus to another. Surface water must be present for this to occur.

20.4 FERNS

The sporophyte generation

In ferns, it is the sporophyte generation that we commonly see. The Christmas fern is a typical example (Fig. 20.2). The **leaves** (or fronds as they are often called) are the only part of the plant that is visible above ground. These arise from an underground stem—the **rhizome**—from which also extend the **roots.** All these structures make up the mature sporophyte generation. During early summer, brownish spots appear on the under side of the leaflets of the fronds (Fig. 20.3). Each dot is called a **sorus** and consists of many sporangia mounted on stalks (Fig. 20.2). Within each sporangium, the spore mother cells undergo meiosis, producing four spores each. When the humidity drops, the thin-walled lip cells of each sporangium separate, and the annulus slowly straightens out. Then with a quick motion, the annulus snaps forward, expelling spores.

You can easily demonstrate this action yourself. Soak a few fern leaflets with ripe sori in a small amount of rubbing alcohol for a day or two. Then scrape some of the sori onto a clean microscope slide with the blade of a knife. Under magnification, the details of the sporangia are easily seen. As the heat from the microscope illuminator passes through the moistened material, the alcohol will evaporate and spore ejection begin.

The gametophyte generation

If the wind-blown fern spores reach a suitable habitat (again moist and shady) they will germinate into filaments of cells. Each grows into a small (¼ inch), flat, green, heart-shaped structure called a **prothallus** (Fig. 20.2). It grows on the surface of the soil anchored by thin filaments of cells called rhizoids. These also absorb water and minerals from the soil. The cells of the prothallus are haploid and it is the mature gametophyte generation. On its under surface are the sex organs: **antheridia** for sperm production and **archegonia** for egg production. When moisture is plentiful, the sperm are released and swim to an archegonium, usually on another pro-

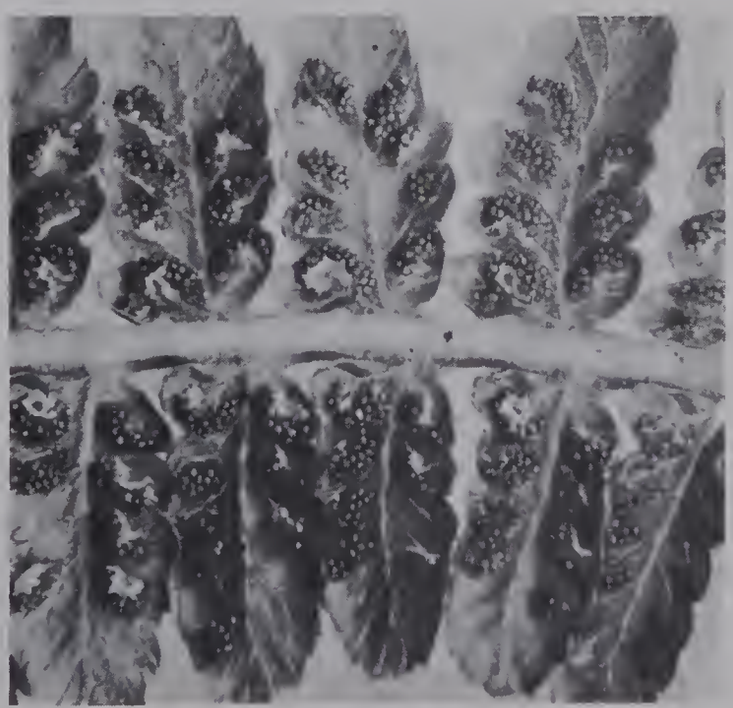


Fig. 20.3 Clusters of sporangia on the underside of fern leaflets.

thallus, because the two kinds of sex organs generally do not mature simultaneously on a single prothallus. This circumstance results in cross-fertilization and thus greater opportunity for variation in the offspring. Fertilization takes place within the archegonium and the new sporophyte generation is initiated. The embryo sporophyte develops by repeated divisions of the zygote. One structure that develops in the embryo but is not found in the mature sporophyte is the foot. This organ penetrates the tissues of the prothallus and derives moisture and nourishment from it until the roots, rhizome, and leaves become self-sufficient. Note that the prothallus, though tiny in comparison with the mature sporophyte, is still an independent, autotrophic plant and even supports the embryo sporophyte during the early stages of its development.

20.5 GYMNOSPERMS

The gymnosperms with which most of us are familiar are the conifers (pines, spruces, etc.) The sporophyte generation (which is the only part the casual observer sees) produces not one, but two, different kinds of spores.

♂ and ♀ gametophytes

There are the small **microspores,** which will germinate to form the male gametophyte generation, and the somewhat larger **megaspores,** which will develop into the female gametophyte generation. Each of these is produced in its own sporangium. In our most common gymnosperms, the conifers, the two kinds of sporangia are produced in the **cones.** Almost everyone is familiar with female cones (Fig. 20.4). The male cones appear in the spring and are much shorter-lived. Within these cones, microspores are produced by meiosis to start the male gametophyte generation. Before being released, mitotic division of the nucleus of each spore takes place with the final production of a four-celled pollen grain. Then the pollen grain is released from the male cone into the air.

The female gametophyte is retained within the parent sporophyte

In the female cones, the megaspore similarly undergoes a period of development within the cone. The product of this is the mature female gametophyte generation (Fig. 20.4). This small structure is not released from the cone but is retained within the tissues of the parent sporophyte. This is seemingly a simple change, but it is one of vast significance to the success of gymnosperms. By retaining the female gametophyte within the tissues of the parent sporophyte, external moisture is no longer necessary for fertilization. The pollen grains are carried by the wind to the female cones. There they germinate. In the earliest gymnosperms, motile sperm were probably released. These could swim to the egg in the archegonium, propelling themselves in fluid supplied by the tissues of the parent sporophyte. This fluid was ultimately derived from a root system extending into the ground. The need for surface water for the sperm was thus eliminated. The ginkgo, commonly planted in city parks, is a gymnosperm that still uses this method of fertilization. For this reason the ginkgo is considered a very primitive type of gymnosperm. In modern gymnosperms, free-swimming sperm are not produced by the male gametophyte. Instead, the pollen grain germinates to form a thin tube, the pollen tube, which grows

Fertilization

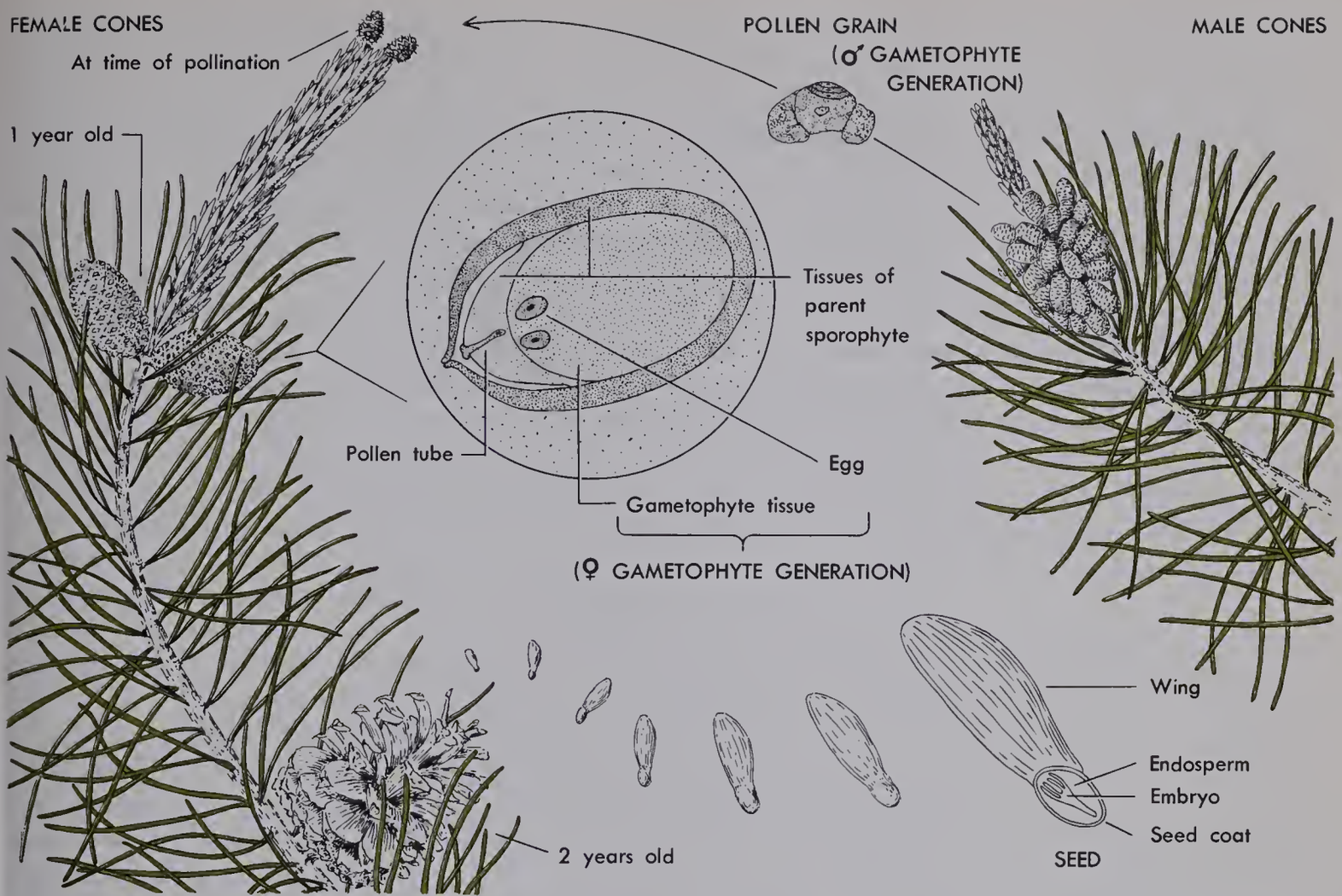


Fig. 20.4 Life cycle of a typical pine. Fertilization of the egg occurs within the tissues of the parent sporophyte.

into the tissues of the female cone until it reaches the vicinity of the egg. (In the pines, this may take a full year.) Then the tube ruptures and a nucleus, the sperm nucleus, fuses with the egg to form the zygote. The basic situation remains unchanged, however. The delicate process of fertilization is carried out deep in the tissues of the parent sporophyte where it is protected from the possible harshness of the external environment.

Gymnosperm spores cannot serve as agents of dispersal

With the production of two kinds of spores and two kinds of gametophytes, spores of the gymnosperms can no longer serve the function of dispersal as they do in mosses and ferns. In mosses and ferns, a single spore can germinate in the *soil* to form a gametophyte with both kinds of sex organs. Sexual reproduction can follow and soon the organism is established in the new environment. Dispersal of the species has occurred. Gymnosperm spores, in contrast, cannot be agents of dispersal. The only place the wind-blown microspore can germinate is on a female cone of the same plant or, better (why?), another plant of the same species. The microspore is simply transported from one plant to another. This does not accomplish dispersal of the species to a new location.

The function of dispersal is taken over by the **seed.** After fertilization, the zygote develops by repeated mitosis into a tiny embryo sporophyte plant (Fig. 20.4). Around the embryo develops accessory food-containing tissue called **endosperm.** It is derived from the cells of the female gametophyte generation and is haploid. Its food came, however, from the parent sporophyte. Around the embryo and endosperm develops a protective coat of parent sporophyte tissue. This coat usually develops a thin vane or wing on one side of the seed. All these developmental activities require another year for completion in the pines. At the end of this period, the female cone opens, releasing, one by one, its contents of seeds. The "wing" of the seed coat serves as a propeller and increases the distance the seed may be carried by the wind. The seed coat itself also serves to protect the embryo from drying out. If the seed is carried to a suitable location (moderately moist), it will absorb water. The embryo starts metabolizing rapidly and then begins to grow. This resumption of growth is called **germination.** At first, growth occurs at the expense of food stored in the endosperm. When the young seedling grows up into the light, however, chlorophyll develops and the plant begins to manufacture its own food by photosynthesis. The endosperm is totally consumed and the seed coat drops away so that every cell of the maturing plant is descended from the zygote.

The seed accomplishes dispersal

Germination of seeds

Compared with the gametophytes discussed earlier, the gymnosperm gametophyte is little more than a reproductory mechanism. Both the male and female gametophytes are tiny and entirely dependent upon the parent sporophyte for nourishment. The gametes can be brought together only through the use of structures of the parent sporophyte generation. The developing embryo is no longer protected by the gametophyte generation as in the mosses and ferns, but receives its nourishment and protection from the parent sporophyte. (The endosperm is gametophytic, but its food stores come from the sporophyte.) The sporophyte generation continues to accomplish dispersal of the species. This is no longer accomplished by wind-blown spores, however, but by wind-blown seeds.

ANGIOSPERMS

The life cycle of the angiosperms, the flowering plants, is similar to that of the gymnosperms. Although minor differences in detail occur in the many species of angiosperms, the major features of the angiosperm life cycle are shared in common.

20.6 THE FLOWER AND ITS POLLINATION

In the angiosperms, microspores and megaspores are produced in flowers. In most angiosperms the flowers are *perfect;* that is, each flower has both microsporangia and megasporangia and thus produces both kinds of

The stamen

spores. The microspores are produced in the **stamens.** The megaspores are produced in the **pistil** (Fig. 20.5). The stamen consists of a lobed structure, the anther or microsporangium, supported by a filament. The pollen grains are formed in the anther. Meiosis of microspore mother cells results in the production of four microspores each. These then develop into a two-celled pollen grain with a tough outer wall.

The pistil

The pistil consists of a stigma, style, and ovary. The ovary contains a chamber within which are located the megasporangia, the **ovules.** The number and arrangement of ovules within the ovary varies greatly from species to species. Meiosis of the megaspore mother cell produces four haploid cells: the large megaspore and three small cells which disintegrate. The nucleus of the megaspore undergoes three successive mitotic divisions. The eight nuclei produced are distributed and partitioned off by cell walls as illustrated in Fig. 20.5. This is the mature female gametophyte generation. The two most importance cells are the **egg cell** and the large central cell which contains the two **polar nuclei.** From this latter cell will be derived the endosperm of the seed.

Although wind transfer of pollen grains is employed by certain groups of angiosperms (e. g. the grasses), the angiosperms are particularly noted for the variety of animals which assist in pollination. Associated with the

Accessory flower parts

stamen and pistil may be found accessory structures. Most obvious of these are the brightly colored **petals** (collectively called the corolla) supported by a whorl of **sepals** (the calyx). Along with the petals may be found glands which emit fragrant odors. It is these accessory structures which give such aesthetic pleasure to us. In woods and fields, gardens and hothouses, the extraordinary variety of corolla shapes and colors are a delight to man. Except for certain domesticated species, however, these shapes and colors do not exist for his pleasure. They serve instead to attract certain animals which, as they pass from flower to flower, may incidentally transport pollen grains from the anther of one to the stigma of another.

Animal-assisted pollination

Animal-pollinated flowers frequently contain nectaries which secrete a sugary solution (nectar) and reward the animal for its visit. Birds (e.g. the hummingbird) and bats pollinate some flowers, but the great majority are pollinated by insects. Beetles, flies, butterflies, moths, and bees are active pollinators. The relationship between the plant and the animal may be quite loose. Some angiosperms are pollinated by many kinds of insects, and some insects pollinate many kinds of plants. In other cases, the relationship is far closer. There is a tropical orchid that is pollinated by just one species of moth, which itself is limited to foraging that one kind of flower for its nectar. The moth has a 10-inch proboscis; the orchid has a nectary 10 inches deep. Other orchids have pistils whose shape and color pattern mimic the abdomens of certain female insects. In attempting to mate with several of these "dummy" females, the male insects effectively transport pollen from one flower to the next.

Generally, insect-pollinated flowers are perfect, both stamens and pistils being found within one flower. Two advantages follow from this.

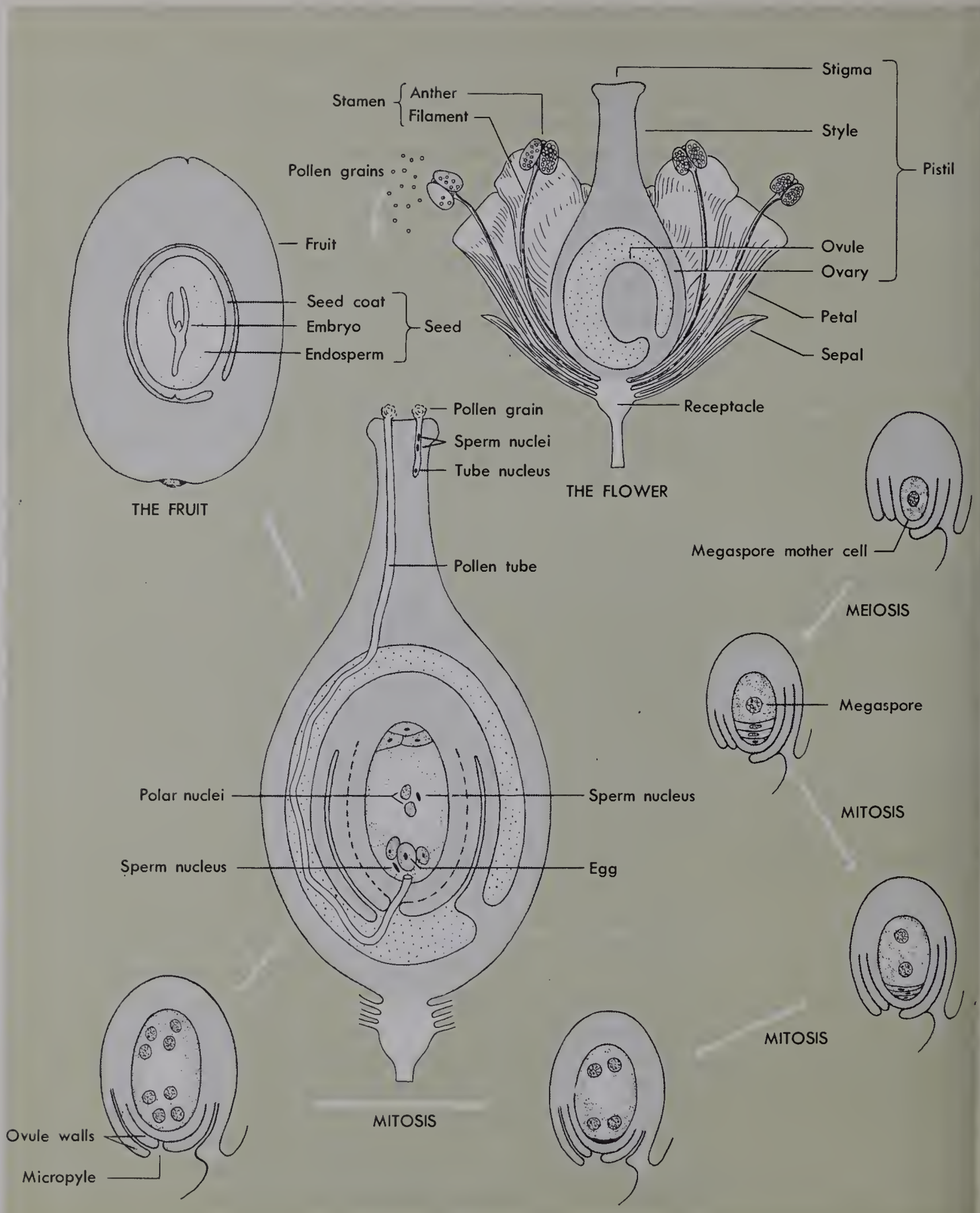


Fig. 20.5 Generalized life cycle of an angiosperm. The fruit develops from the wall of the ovary.

Perfect vs. imperfect flowers

First, there is a greater likelihood of pollination occurring. At each visit by the pollinator, pollen from the last flower is deposited while fresh supplies of pollen are picked up. With **imperfect flowers,** which contain either stamens or pistils but not both, a pollinating insect has to visit staminate flowers and pistillate flowers alternately in order to achieve a comparable efficiency as a pollinator. Second, if pollination between different flowers (cross-pollination) should fail to occur, the flower may still be able to pollinate itself. Seed production will still occur, although the similar heredity of the gametes will diminish the amount of variability in the offspring.

Self-pollination

The flowers of some angiosperms are actually modified in such a way that self-pollination is the rule rather than the exception. As we noted in Chapter 17 the corolla of the common pea completely encloses the stamens and pistil so that insects cannot easily reach these structures. Many violets produce, in addition to their conspicuous cross-pollinated flowers, smaller flowers that never open to expose their stamens and pistil to insect pollinators. A glance at Fig. 20.6 will show why only self-pollination occurs in these flowers.

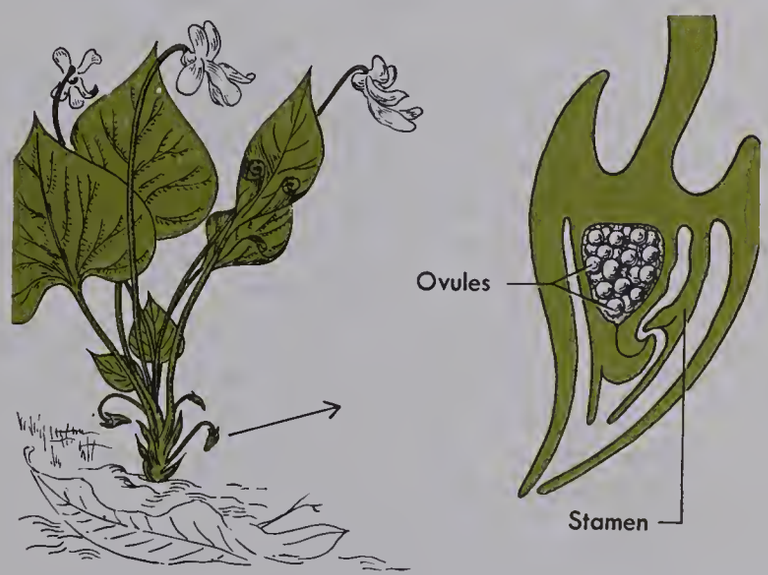


Fig. 20.6 Violet flowers. The petal-bearing flowers are cross-pollinated. The others (enlargement) are self-pollinated.

Promoting cross-pollination

Flower modifications to promote cross-pollination are more common than those to prevent it. In many species the stamens and pistil of a single flower mature at different times. In some species the floral parts are arranged so that there is little likelihood of a direct transfer of pollen from anther to stigma. The sage (*Salvia*) flower embodies both these mechanisms in addition to an unusual hinged stamen which bends downward to dust pollen on the bee which triggers it (Fig. 20.7). In many angiosperms, e.g. red clover and some apple varieties, pollen will not germinate on a stigma of the same plant. These plants are called self-sterile.

Dioecious vs. monoecious plants

Still another mechanism that ensures cross-pollination is the occurrence of imperfect flowers on separate plants. Species in which this occurs, such as the willows, poplars, and the date palm, are called **dioecious.**

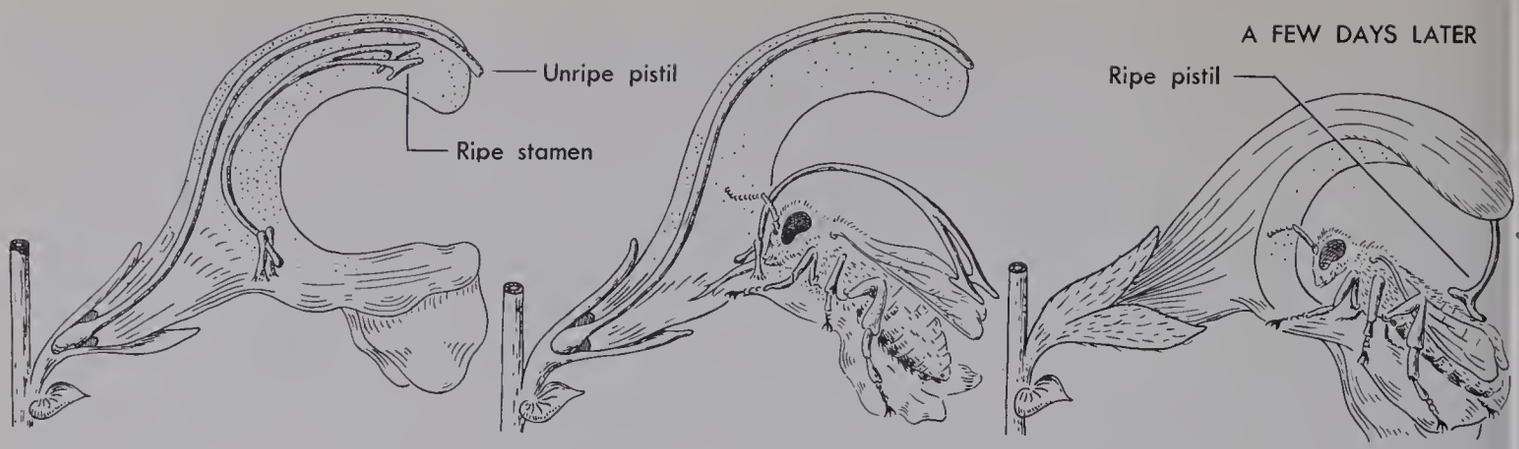


Fig. 20.7 Pollination in *Salvia.* Cross-pollination is assured by (1) ripening of the stamen before the pistil, (2) a trigger mechanism that dusts pollen on the pollinator, (3) later growth of the pistil so that it brushes the bees that continue to forage the flower for nectar.

(Species with imperfect flowers on one plant are called **monoecious.** Fertilization between these flowers produces no more hereditary variability than self-pollination within a single flower.)

In one locality all the plants of a given species flower at roughly the same time. This usually occurs even if the various individual plants have begun growing at different times. As we shall see in Chapter 24, such synchronous flowering seems to be a response to the changing length of day and night as the season progresses. It, too, is an important factor in encouraging cross-pollination.

Wind pollination

Our emphasis on animal-pollinated angiosperms should not obscure the fact that many angiosperms are wind-pollinated. Poplars, oaks, elms, birches, plantains, ragweed, grasses, and many others are wind-pollinated. Their flowers are not apt to have petals, odor, or nectar. They are frequently imperfect (as are the cones of gymnosperms which, you remember, are also wind-pollinated). The stamens of the staminate flowers are exposed to the wind and produce large quantities of light, dry pollen. This wind-blown pollen may cause hay fever in allergic humans. The stigmas of the pistillate flowers are often long and sticky. The ovary usually contains only a few ovules.

Although the wind-pollinated flowers do not provide much aesthetic pleasure for man, they are largely responsible for man's ability to find time for any aesthetic pleasures at all. All our cereal grains come from wind-pollinated species. As a group, they provide, directly or indirectly, the major portion of the food consumed by mankind.

Fertilization

When, by one means or another, the pollen grain reaches the stigma of a flower of the same species, it germinates into a pollen tube. The pollen tube contains two nuclei: the tube nucleus and the generative nucleus. As the pollen tube starts to grow down the style into the ovule chamber, the generative nucleus divides by mitosis, forming two sperm nuclei. The pollen tube, with its three nuclei, is the mature male gametophyte genera-

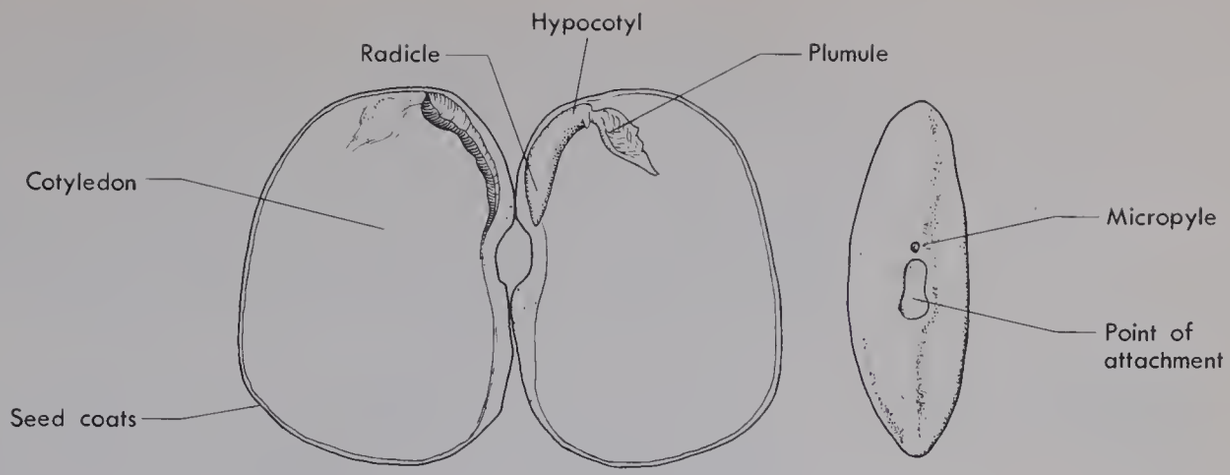


Fig. 20.8 Structures of a typical dicot seed, the bean.

tion. It enters the ovule through the *micropyle* (Fig. 20.5) and ruptures. One sperm nucleus fuses with the egg, forming the zygote ($2n$). The other sperm nucleus fuses with two polar nuclei to form an endosperm nucleus containing $3n$ chromosomes. The tube nucleus disintegrates.

20.7 THE SEED

Mitotic divisions of the zygote and endosperm nucleus result in the formation of the seed (Fig. 20.8). It consists of:

1. A **plumule,** made up of two embryonic leaves, which will become the first true leaves of the seedling, and a terminal (apical) bud. The terminal bud is the meristem at which further growth of the stem will occur.
2. The **hypocotyl** and **radicle,** which will grow into the stem and primary root, respectively.
3. The **cotyledons,** which store food that will be used by the germinating seed. This food is derived from the endosperm which, in turn, received it from the parent sporophyte. In many angiosperms (the common bean is one example), the endosperm has been totally consumed and

Fate of the endosperm

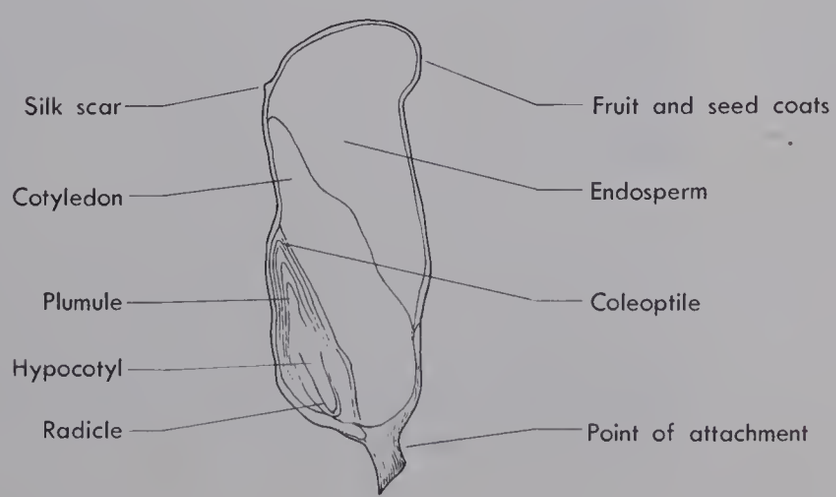


Fig. 20.9 Structures of a corn kernel. Because its outer covering is derived from the ovary wall of the flower, the corn kernel is actually a fruit with a single seed inside.

its food stores transferred to the cotyledons by the time development of the seed is complete. In others, the endosperm persists in the mature seed. This is true of some dicots and all monocots. The latter, of course, have only one cotyledon in the seed (Fig. 20.9). The cells of the endosperm are triploid ($3n$) in contrast to the haploid (n) endosperm of the conifers and other gymnosperms (see Section 20.5). While these structures are developing, the walls of the ovule thicken to form the protective seed coats.

Functions of the seed

The seed is thus a dormant embryo sporophyte with stored food and protective coats. Its two functions are: (1) dispersal of the species to new locations and (2) maintenance of the species over periods of unfavorable climatic conditions. "Annuals" (such as the bean, cereal grains, and many weeds) can survive as a species only by the production of resistant seeds. With the onset of freezing weather in the fall, the mature plants die. However, many of their seeds will remain alive, though dormant, throughout the winter. When conditions once more are favorable for the growth of the plant, germination of the seeds occurs and a new generation of mature plants develops.

20.8 SEED DISPERSAL: THE FRUIT

The fruit is a development of the ovary wall and sometimes other flower parts as well. It contains the seeds. To a biologist, the term *fruit* is not restricted simply to those succulent types which man enjoys eating. Figure 20.10 shows some of the many different types of fruits produced by angiosperms.

Wind dispersal

In every case these fruits aid in the dispersal of their content of seeds. The maple "key" and the dandelion parachute are examples of fruits which facilitate dispersal of the seeds by the wind. The cocklebur and sticktights are fruits which achieve dispersal of their contained seeds by sticking to the coat (or clothing) of a passing animal. The animal's power of locomotion serves to transport the seeds to new locations.

Water dispersal

The coconut is a fruit which achieves despersal of its single seed by *floating* to new locations. Groves of coconut palms ring all the Pacific islands. Trees leaning out over the water may drop their coconuts in. The fruit does not germinate in salt water but it will remain viable (capable of germinating) for long periods as it is carried by ocean currents. Finally cast up on a beach, it will be washed by rain water and may germinate and develop into another mature palm.

Mechanical dispersal

Some of the most interesting fruit adaptations are those which achieve mechanical dispersal. Legume pods (e.g. wisteria), witch-hazel pods, touch-me-nots and the squirting cucumber all forcibly eject their seeds as the fruit dries out in the fall. While the distances traveled by these seeds do not achieve rapid dispersal of the species, they do decrease the likelihood of the seedlings having to compete with the parents for sunlight, moisture, and soil minerals.

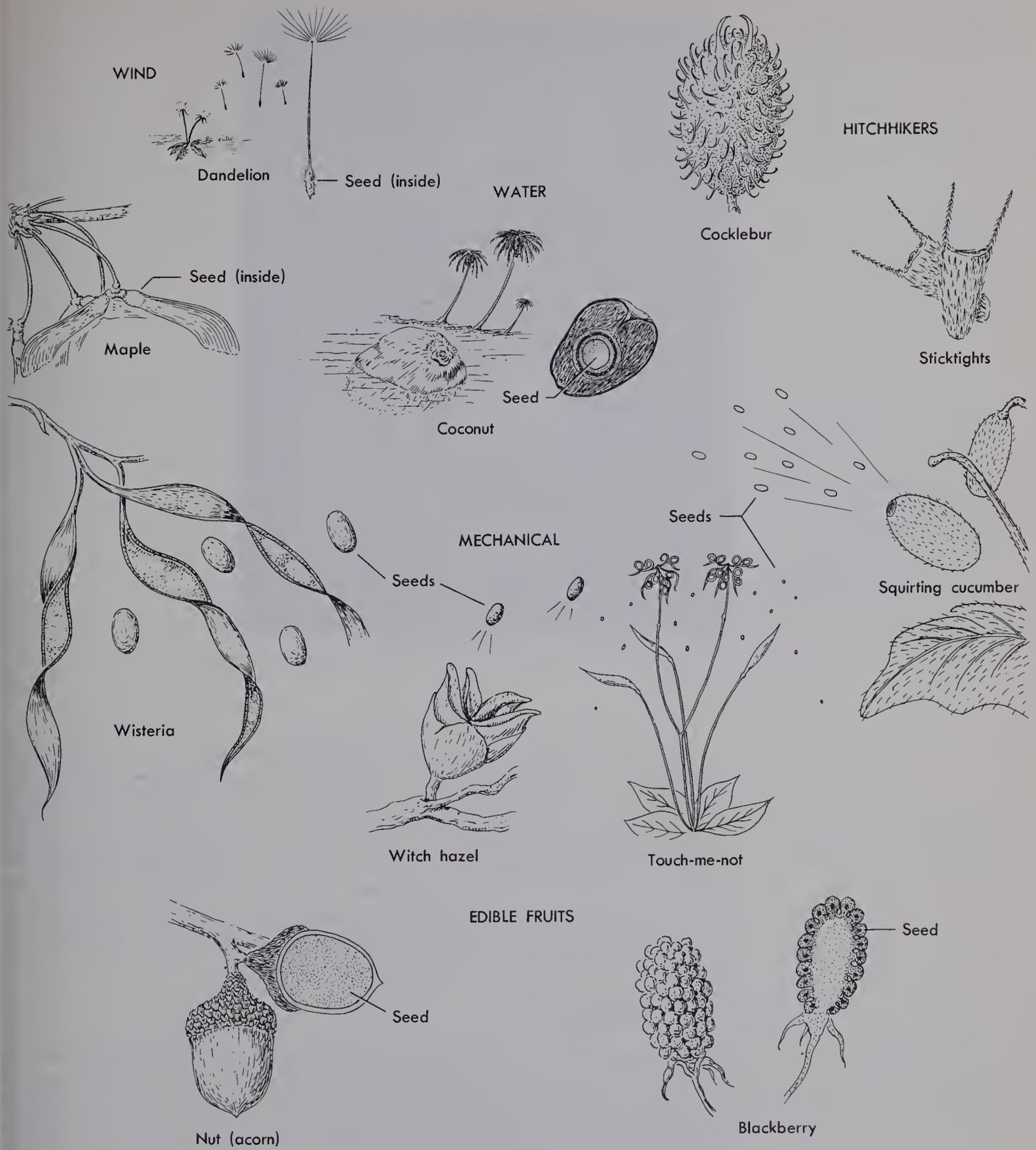


Fig. 20.10 Fruits and their role in seed dispersal.



Fig. 20.11 Three stages in the germination of a bean seed, shown by time-lapse photography. The cotyledons protect the plumule from damage as it is lifted through the soil. (Courtesy of the Pittsburgh Plate Glass Co.)

Animal-assisted dispersal

Edible fruits are very effective dispersal mechanisms. Squirrels, field mice, and other rodents seldom eat all the nuts and grains they store in the fall. With the coming of spring, the forgotten seeds may germinate and at considerable distances from the parent plant. The various kinds of berries are characterized by tiny seeds which can pass undamaged through the alimentary canal of the bird or other animal that eats them. When the animal expels the seeds in defecation, it is usually in quite a new location. The manure in which the seeds are defecated serves as a rich source of minerals and humus for the developing seedling.

20.9 GERMINATION

Requirements for germination

Germination is the resumption of growth of the embryo plant inside the seed (Fig. 20.11). Proper temperature, proper amounts of moisture, and an adequate supply of oxygen are all necessary for it to occur. What is proper or adequate for one species may not be for another, but for each species these three conditions must be met to some degree.

A period of dormancy is also a requirement for the germination of many seeds. For example, the seeds of apples and peaches will germinate only after a prolonged period of cold. There is evidence that a chemical inhibitor is present in the seed when it is first formed. This inhibitor is

gradually broken down at low temperatures until finally there is no longer enough to prevent germination when the other conditions become favorable. Can you think of any survival value this mechanism may have for the plant? The seeds of many desert angiosperms possess inhibitors which prevent germination until the substance has been leached away by water in the soil. In this process more water is required than is necessary for germination itself to occur.

Exposure to light of proper duration is also a condition for germination in some cases. The seeds of some plants which grow in marshy locations will germinate only after prolonged exposure to light. On the other hand, the germination of the seeds of some desert plants is inhibited by prolonged exposure to light. What survival value might these responses have?

Summary

The angiosperm life cycle is basically similar to that of the gymnosperm. Like that of the gymnosperm, it permits successful existence in a terrestrial environment. The angiosperms, however, are a far more varied and successful group than the gymnosperms. There are approximately 200,000 species of angiosperms today compared with approximately 650 species of gymnosperms. Further, the angiosperms have exploited far more kinds of habitat than have the gymnosperms. Arctic tundra, plains, temperate forests, deserts, and jungles are types of environment where angiosperms grow in far greater variety and numbers than do the gymnosperms. The greater abundance and variety of angiosperms and their wide distribution throughout the world is evidence of the survival value of their two unique structural adaptations: the flower and the fruit.

EXERCISES AND PROBLEMS

1. Where are the sporangia located in the (a) moss, (b) fern, (c) pine, (d) apple tree?
2. In what ways are the gametophyte generations of the moss, fern, and angiosperm alike?
3. In what ways are they different?
4. In what ways are the sporophyte generations of the moss, fern, and angiosperm alike?
5. In what ways are mosses well adapted to life on land? In what ways are they poorly adapted?
6. What three conditions are always necessary for germination of seeds to occur?
7. What additional factors are sometimes necessary for the germination of seeds?
8. Describe the experimental procedure that you would use to test the viability of bean seeds that had been stored for five years.
9. Describe the experimental procedure you would use to determine the optimum temperature for the germination of bean seeds.
10. What plant tissue is normally triploid?
11. Which cells undergo meiosis in the (a) moss and (b) bean?

12. Reproduction in the closed flowers of the violet (see Fig. 20.6) is sexual because it involves the union of two gametes. Does it accomplish what sexual reproduction in the open flowers does? What would be the long-term consequence if the violet reproduced exclusively by means of its closed flowers?
13. In angiosperms, what do the ovules eventually become? What does the ovary become?
14. Summarize the adaptions found in angiosperms that prevent or minimize self-pollination.

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21 SEXUAL REPRODUCTION IN ANIMALS

The Formation of Gametes

Bringing the Gametes Together

Fertilization

Care of the Young

Human Reproduction

The Sex Organs of the Male

The Sex Organs of the Female

Fertilization, Pregnancy, and Birth

Reproductive Engineering: the Prospects

21.1 THE FORMATION OF GAMETES

Animals, unlike plants, do not exhibit an alternation of diploid and haploid generations. Fertilization is still preceded by meiosis, but the products of meiosis are the gametes themselves. In all animals, **heterogametes** are produced. A moment's reflection will reveal the adaptive value of this modification. To carry out their function most effectively, gametes should be motile (so they can meet and unite) and supplied with food reserves to

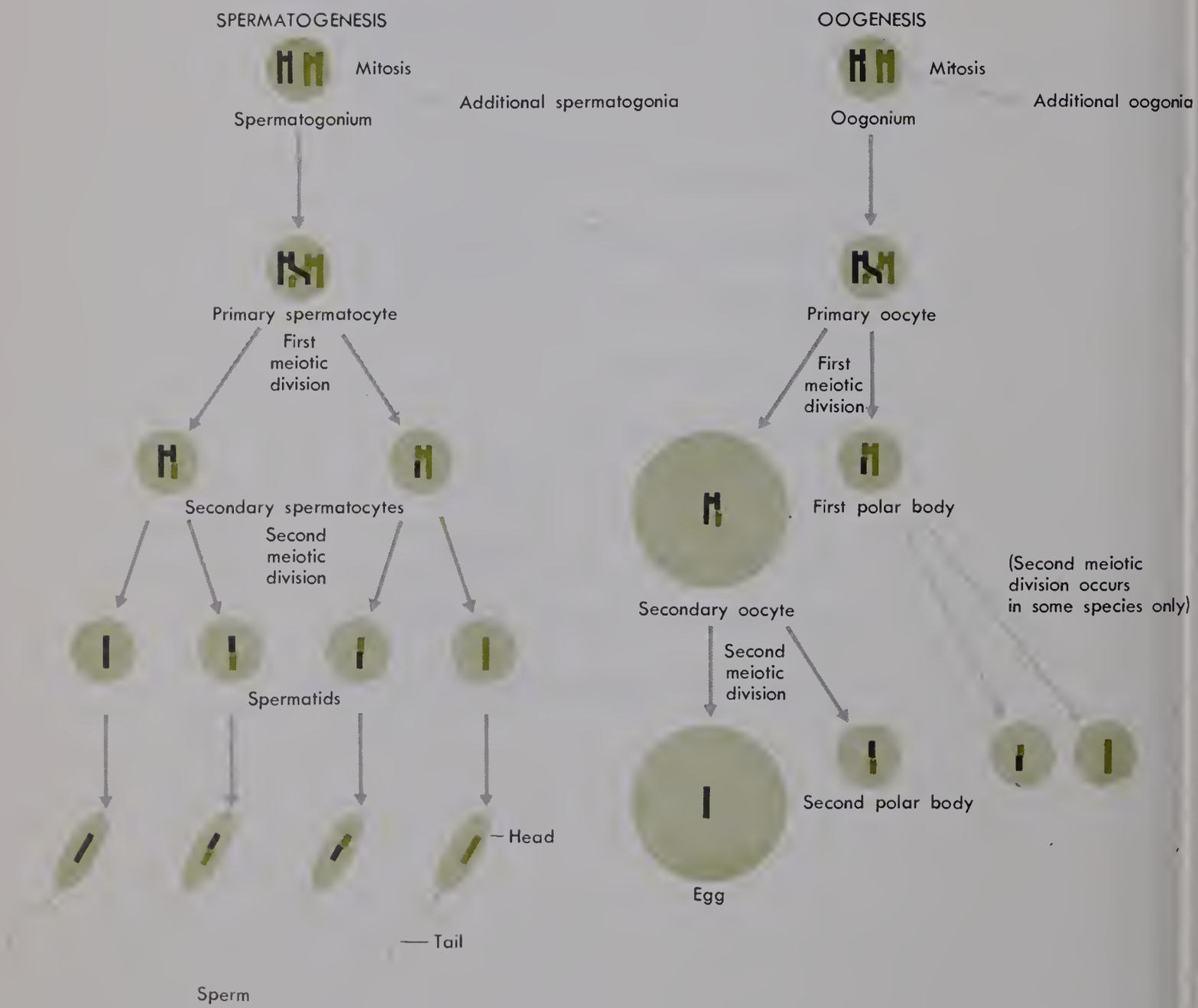


Fig. 21.1 Spermatogenesis and oogenesis. For the sake of simplicity, the behavior of just a single pair of homologous chromosomes is shown (cf. Fig. 16.12). With two or more pairs, random assortment of the homologues contributes to the variety of gene combinations in the gametes. Note that which chromosome ends up in the egg and which in the polar bodies is entirely a matter of chance.

Sperm and egg

furnish energy and material for the developing embryo. These two requirements are rather incompatible. The solution is: one gamete, the **sperm,** that is motile and small and one gamete, the **egg,** that is filled with food reserves.

Spermatogenesis

Sperm cells are little more than flagellated nuclei. They are produced in **testes** by specialized cells called spermatogonia. Spermatogonia, which are diploid, may divide by mitosis to produce additional spermatogonia or they may be transformed into *spermatocytes.* Meiosis of each spermatocyte results in the production of four haploid cells, the spermatids (Fig. 21.1). These then become transformed into sperm cells, losing most of their cytoplasm in the process.

A sperm cell consists of (1) a head, which contains the chromosomes in a compact, inactive state, (2) two centrioles, and (3) a "tail." One of the centrioles will help to construct a spindle for the sperm and egg chromosomes following fertilization. The other serves as the basal body of the flagellum, which extends the length of the tail. Mitochondria surround the upper part of the flagellum and supply the energy for its lashing movements (Fig. 21.2).

Eggs are produced in **ovaries.** Diploid oogonial cells divide by mitosis to produce additional oogonial cells. This occurs once a year in most



Fig. 21.2 Sperm cell of a bat (15,000 X). Note the orderly arrangement of the mitochondria in the tail. They supply the energy for its motion. (Courtesy of Don W. Fawcett and Susumu Ito.)

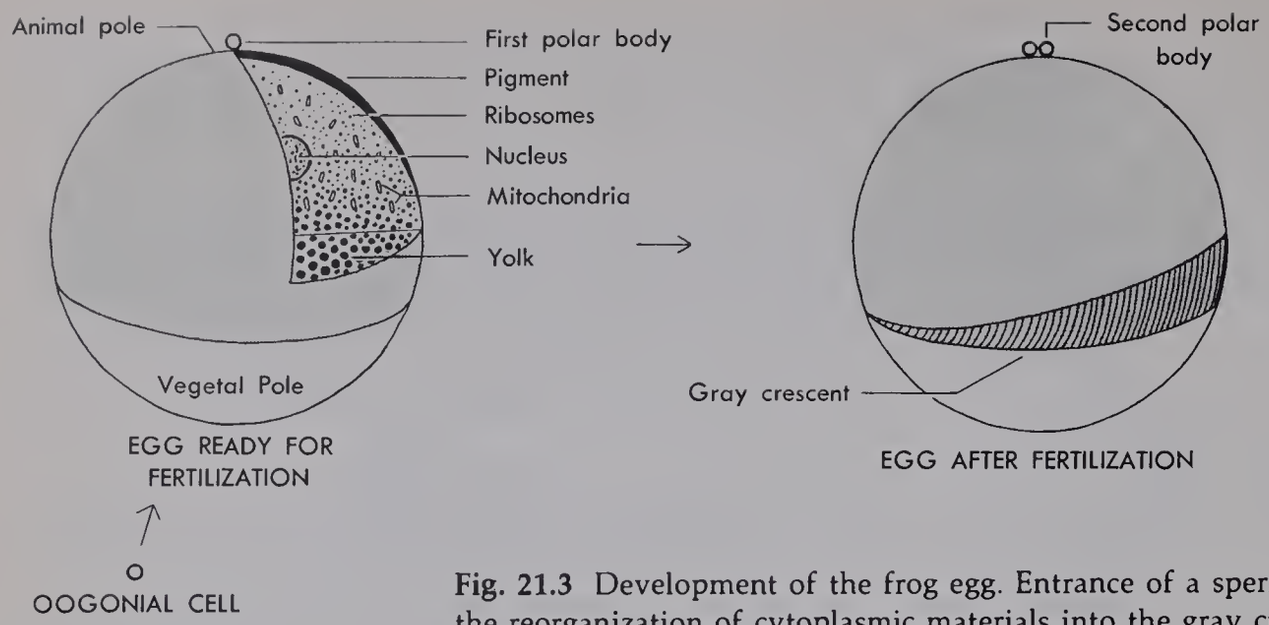


Fig. 21.3 Development of the frog egg. Entrance of a sperm cell is followed by the reorganization of cytoplasmic materials into the gray crescent and then the completion of the second meiotic division.

Oogenesis

aquatic animals and in the amphibians. Among the reptiles, birds, and mammals, however, the process stops long before birth. In fact, by the time the female human fetus (a developing baby) is 15 weeks old, multiplication of oogonia is almost completed. This certainly justifies Weismann's emphasis on the early isolation of the germplasm from the somaplasm!

The frog egg

The beginning of egg formation occurs when oogonia start growing and become transformed into primary oocytes. These diploid cells enter prophase of the first meiotic division and at that point their development stops. No further development of the primary oocytes takes place until just before the animal is ready to enter a period of reproductory activity. In frogs, this occurs once a year—usually in the spring—after adulthood is reached. Then thousands of primary oocytes begin a period of marked cell growth. Each is enclosed in a cluster of cells called a **follicle.** Food materials are transferred from the follicle cells to the growing oocyte. The volume of frog eggs increases over a million times during this period (Fig. 21.3).

When this phase of development is completed, the egg cell is a large sphere containing in its cytoplasm large quantities of DNA, RNA, yolk, mitochondria, and oil droplets. These materials are not distributed uniformly throughout the egg of the frog but in gradients extending from pole to pole. The dark hemisphere of the egg is topped by the so-called **animal pole.** Except for the yolk, most of the egg constituents are concentrated near this pole. The nucleus is also found here. The yolk concentration increases toward the opposite, light-colored **vegetal pole.**

Ovulation

As growth of the primary oocyte nears completion, the first meiotic division is completed, too. The cytoplasm is not distributed equally to the two daughter cells, but almost all of it passes to just one of them. The other cell is called a **polar body** (Fig. 21.4).

In most vertebrates, the second meiotic division gets only as far as metaphase and then stops. At this time, the egg is ready for release from



Fig. 21.4 Polar body formation during oogenesis in the whitefish. (Courtesy of the General Biological Supply House, Inc.)

the follicle, a process known as **ovulation.** The follicle and ovary walls rupture, allowing the egg to pass into the body cavity. It then enters an oviduct where accessory substances, such as gelatinous rings of albumen in the frog, may be added. Further development of the egg awaits fertilization.

The life span of liberated sperm and eggs is usually measured in hours. Furthermore, sperm are definitely limited in the distance they can swim. But, since most animals have the power of locomotion, the sperm themselves do not usually have to travel great distances to reach the egg. Successful locomotion of the sperm and successful fertilization do, however, require moist surroundings.

21.2 BRINGING THE GAMETES TOGETHER

Aquatic animals

Among aquatic animals, the problem of moist surroundings for fertilization does not exist. Fertilization can occur directly in the water after each parent releases its gametes. To improve the chances of the gametes meeting, they are usually released in fairly close proximity. This requires that the males and females of the species reach full sexual development at about the same time and in about the same place. Synchronous flowering achieved by response to definite photoperiods was discussed in the last chapter. Analogous periodic behavior is observed in many animals. In the California grunion (a fish), the two sexes swim into shallow water to spawn (release their gametes) at the time of full moon and new moon. In other species, a member of one sex (usually the male) carries out certain behavioral activities ("courtship") in the presence of the female. These activities trigger appropriate egg-laying behavior in the female.

Terrestrial invertebrates

The first terrestrial animals were probably scorpions and insects. Life on land created a problem in bringing the gametes together while protecting them from the drying action of the air. It was solved by internal fertilization. A special organ of the male, the penis, is inserted into the

female in a process called **copulation** and transfer of the sperm takes place. In insects, the sperm are then stored in sperm receptacles until the female is ready to lay her eggs. As the eggs pass down her genital tract, they are fertilized by the sperm. The critical process of fertilization is thus accomplished entirely within the moist body of the female (Fig. 21.5).

Amphibians

The first terrestrial vertebrates were the amphibians. As the name implies, they are really only semiterrestrial. Most frogs and toads must return to the water to engage in sexual reproduction. Fertilization takes place in the water. The common male bullfrog, for example, clasps the female in an embrace called amplexus. As the female deposits her several thousand eggs into the water, the male deposits sperm over them. No copulation takes place.

Reptiles, birds, and mammals

The reptiles were the first vertebrates able to live in a truly terrestrial environment. They have several important adaptations, both structural and physiological, which permit them to flourish even in hot, dry climates. Some of these will be discussed in Chapter 37. As for sexual reproduction, the problem of moisture for the gametes is solved by internal fertilization. In most cases, the male copulates with the female, introducing sperm directly into her genital tract. Birds and mammals also solve the problem of bringing the gametes together in moist surroundings by copulation followed by internal fertilization.

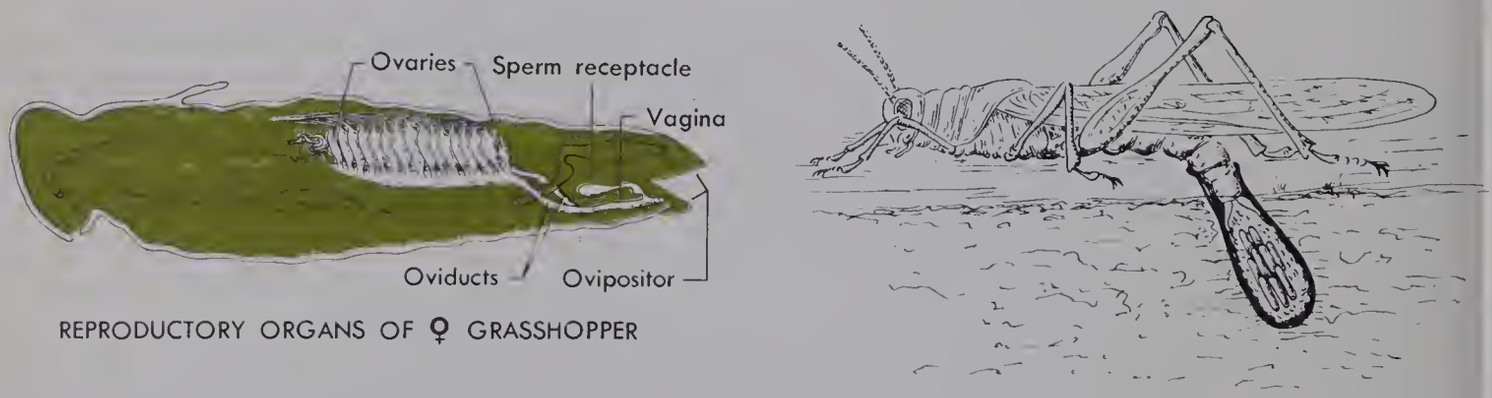


Fig. 21.5 Reproductory organs of the female grasshopper. Sperm are stored in the sperm receptacles following copulation with the male. Fertilization takes place as the eggs pass through the vagina before being deposited in the soil.

21.3 FERTILIZATION

Entrance of the sperm

The process of fertilization begins when sperm actually become attached to the egg. In so doing, they may release digestive enzymes that dissolve an opening through the membrane or layer of residual follicle cells that usually cover eggs. Then the nucleus and one centriole of the sperm cell actually enter the egg. The egg seems to play an important part in this as these structures appear to be drawn inward. Entrance of the sperm head is followed by a rapid and dramatic transformation within the egg itself.

Its cytoplasmic constituents quickly become reorganized. In the frog, certain cytoplasmic granules appear at the surface of the egg in a band called the **gray crescent** (Fig. 21.3).

In many eggs, the changes brought about by the entrance of one sperm immediately prevent, in some way, other sperm from entering, too. Just how this is accomplished is not fully understood. In animals with large eggs, such as reptiles, birds, and the duckbill platypus, it doesn't occur at all. Although many sperm gain entrance to these eggs, only one contributes its nucleus to the future zygote. In any case, entrance of a sperm cell also triggers the completion of the second meiotic division, and a second polar body forms.

Formation of the spindle

The final event in fertilization occurs when the sperm centriole initiates the formation of a spindle upon which first the sperm chromosomes and then the egg chromosomes become arranged. At this point, a zygote with the diploid number of chromosomes has been formed and fertilization is complete. Within a short time, the first mitotic division of the cell will take place and embryonic development begin.

21.4 CARE OF THE YOUNG

An important relationship exists between the number of gametes, especially eggs, produced by different animals and the care given the embryos during development. For a population to continue at a stable size, an average of two offspring should reach maturity for every two parents. Very few aquatic animals take any care of their eggs once they are fertilized, and mortality of the developing young is extremely high. In compensation for this, a mature female American oyster, *Crassostrea virginica,* may release as many as one million eggs per season. The chances of any one egg ever developing into a mature oyster are exceedingly small. Most, even if successfully fertilized, will succumb at some stage of development to predators, infection, or other unfavorable happenings. Some aquatic animals, e.g. many freshwater bivalves and some fish, do provide a certain amount of care for the eggs. The freshwater clam retains fertilized eggs in the gills until they hatch. The male stickleback fish builds a nest and guards and aerates the eggs that the female deposits inside. Characteristically, these species do not produce such enormous numbers of eggs.

Aquatic animals

The eggs of most aquatic animals are relatively small. In a large number of species, the organism that hatches from the egg is not a miniature replica of the adult but a larval stage quite different in appearance. Usually the larvae are free-swimming and feed on miscroscopic plant and animal life called plankton. In so doing, they acquire additional food materials for further growth. After a period of growth, the larvae undergo metamorphosis, and the body structure is reorganized on the plan of the adult. Metamorphosis may occur in several stages (e.g. in crustaceans and mollusks) or it may be completed in a single brief, but extensive, transformation (e.g. in the starfish).

Larvae

The cartilaginous fishes

An exception to these generalizations on aquatic animals is found in the cartilaginous fishes: the sharks, skates, and rays. Their interesting solution to the problem of dehydration by salt water was discussed in Chapter 15. You remember that the concentration of urea in the bloodstream is maintained at such a high level that the blood is isotonic to the sea water. This physiological adaptation cannot be achieved by their embryos. The cartilaginous fishes have, however, developed two techniques for avoiding fatal dehydration of their embryos. One is to enclose the fertilized egg and a suitable watery medium in a tough, waterproof case. Empty skate egg cases are a familiar sight among the debris washed up on our beaches (Fig. 21.6). The other solution is to retain the developing embryo within the female's body, as some sharks do, until it becomes physiologically capable of maintaining a high urea level. The young then are "born." Either solution requires internal fertilization of the eggs. (The egg case of the skate is as impervious to sperm as it is to water.) The sperm are introduced directly into the body of the female in the process of copulation. Modified fins on the male aid in clasping the female and in transferring the sperm.

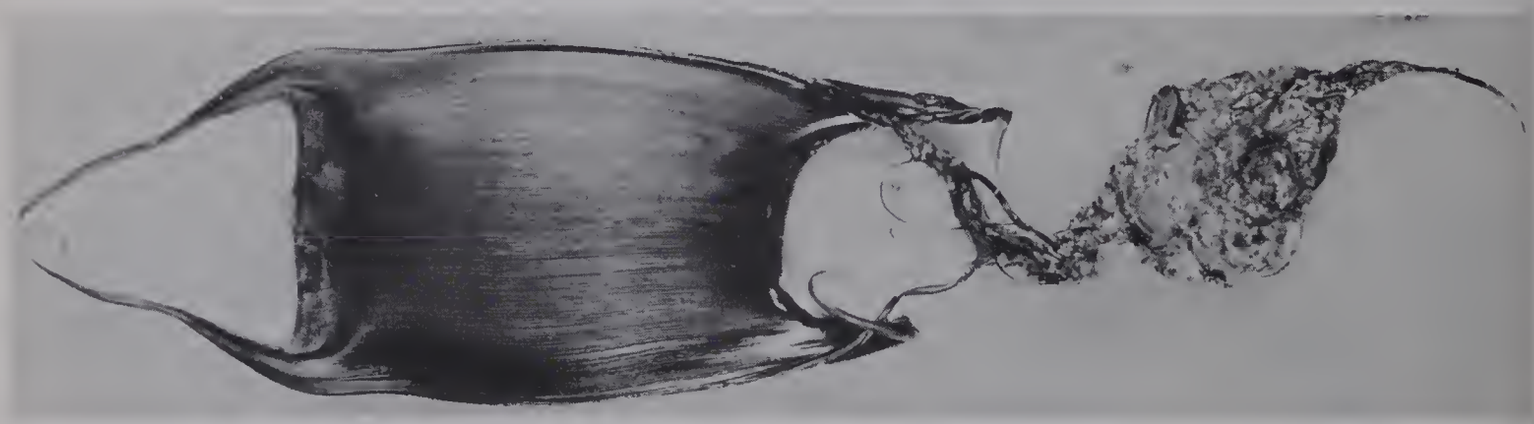


Fig. 21.6 Egg case of a skate (almost life-size). The eggs are sealed inside. (Courtesy of the New York Zoological Society.)

Insects

Terrestrial animals all need some means of protecting the developing embryo from the drying action of the air. In insects this protection is provided by a waterproof covering deposited around each egg and, in some cases, a capsule or sac which is deposited around a cluster of eggs. The number of fertilized eggs laid each season varies from hundreds to thousands, depending on the particular species and the amount of care taken of the eggs. The smaller numbers reflect a somewhat greater chance of survival to adulthood for a given embryo than in the aquatic species discussed earlier. Care of the young is developed to an extreme in colonial ants and bees. In these species a very high percentage of the fertilized eggs develops to maturity. Significantly, most of the offspring of these species are sterile workers, which makes the maintenance of a stable population easier.

Amphibians

Most of our common amphibians take no care of their eggs. After fertilization, they are simply left to develop in the water. The gelatinous layers of albumen surrounding the egg absorb water, swell, and provide some physical protection for the egg. They also aid in keeping the egg somewhat warmer than the surrounding water. The clear jelly is transparent to the visible portion of the sun's rays. The dark upper surface of the developing egg absorbs these rays effectively. The light energy is converted into heat which warms the embryo. The jelly is, however, a good heat insulator and less energy radiates from the embryo than was received by it. This physical phenomenon is familiar to anyone who has entered an automobile that has been standing in the sun for some time with the windows closed.

The dark upper surface of the fertilized egg also provides protective coloration. When viewed from above, the egg is hard to distinguish against the dark background of the pond bottom. Similarly, the light underside of the egg blends with the sky when viewed from underneath.

Reptiles

Reptile eggs are fertilized in the genital tract of the female. Then a waterproof shell is deposited around each as it passes down the oviduct. This solves the problem of maintaining the embryo in moist surroundings after the egg is laid. In most species, the eggs are buried in a warm location and abandoned by the mother. There is a plentiful supply of yolk and when the young hatch, they are replicas, although smaller, of their parents. They are also quite self-sufficient.

In some reptiles, e.g. the common garter snake, the eggs are retained in the mother's body until the young hatch. Their nourishment throughout this period is derived chiefly from the yolk of the egg rather than directly from the mother's tissues.

Not long after reptiles first appeared on earth, some species returned to an aquatic environment. Today, one species of lizard (the marine iguana), some snakes and turtles, and the various crocodilians spend most of their lives in water. Nevertheless, those that lay their eggs return to land to do so.

A loggerhead turtle lays about 125 eggs in a season. Although left unprotected by the parents, the mortality rate is sufficiently low so that the population is maintained. Some reptiles are among the most long-lived animals on earth. An old female turtle has had a good many opportunities to contribute to the maintenance of the population.

Birds

Birds are also fully adapted to life in dry locations. Like many of the reptiles (from which they are descended), they lay shelled eggs. These are often carefully tended by the parents. The eggs are richly supplied with yolk, and the young hatch with most of the adult structures. The parents continue caring for the newly hatched young until they are ready to care for themselves. Appropriately, birds rarely lay more than 20 eggs in a season and the average is probably closer to four.

The shelled egg is obviously an important adaptation permitting bird and reptile embryos to develop away from water. A close examination of a common bird egg, the hen's egg, will reveal why this is so. The egg itself

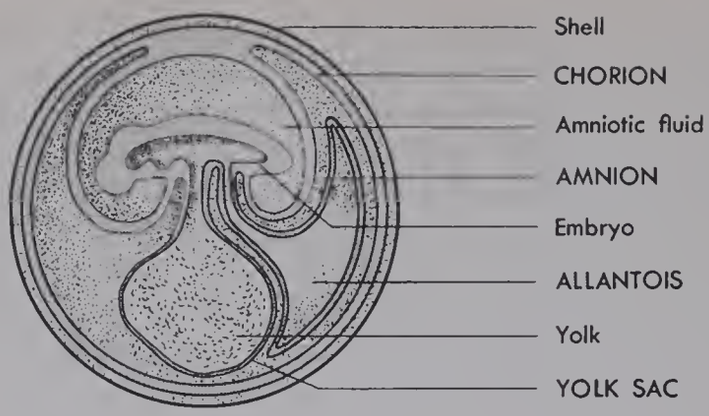


Fig. 21.7 The extra-embryonic membranes of the developing chick. Note that the yolk sac and allantois are outgrowths of the embryo digestive tract. The same membranes are produced by the embryos of reptiles and mammals.

consists of a large quantity of yolk and a tiny bit of cytoplasm. After fertilization, but while still within the mother's oviducts, the egg is surrounded with thick layers of watery albumen (egg "white") and a shell made of calcium carbonate. The shell is permeable to gases, but practically impermeable to water. As the embryo develops from the zygote, four special membranes are formed (Fig. 21.7). These "extra-embryonic" membranes develop from the embryo but remain part of it only during its existence within the egg. These four membranes are:

The extra-embryonic membranes

1. The **yolk sac,** which surrounds the yolk. It connects the embryo with its major source of food. Fats constitute most of the food in the yolk. This is a valuable adaptation, since the oxidation of fats supplies large amounts of both energy and water.
2. The **amnion,** which grows around the embryo, enclosing it in a fluid-filled cavity.
3. The **chorion,** which lines the inner surface of the egg shell. It aids in the exchange of gases (O_2 and CO_2) between the embryo and the outside air.
4. The **allantois,** which serves as a reservoir for the metabolic wastes (chiefly uric acid) excreted by the embryo during its development. As the allantois grows larger, it also participates in gas exchange.

With these four membranes, the developing embryo is able to carry on essential metabolism while sealed within the egg. Surrounded by the fluid in the amniotic cavity, the embryo is kept as moist as the fish embryo in a pond.

The echidna and duckbill platypus lay eggs

These four membranes are produced by the embryos of birds, reptiles, and also mammals. Except for the rare echidnas and duckbill platypus, though (Fig. 21.8), the membranes are not associated with a shelled egg in the mammals. Mammalian eggs, which are poorly supplied with yolk, are retained within an oviduct after fertilization. The extra-embryonic membranes penetrate the walls of an enlarged portion of the oviduct, the **uterus.** After the small supply of food within the egg is exhausted, additional nourishment is secured through exchange with the mother's circulatory system.



Fig. 21.8 Mammals that lay eggs. The duckbill platypus (left) raises its young in a nest; the spiny anteater (right) places them in a pouch on its abdomen. (Courtesy of the New York Zoological Society.)

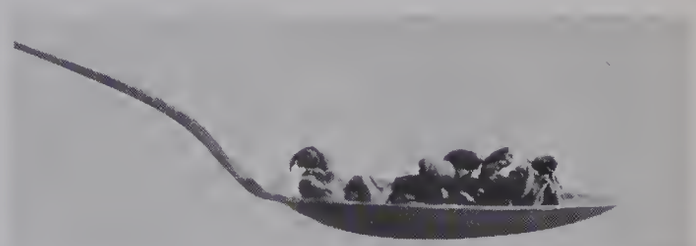


Fig. 21.9 Newborn opossums. Eighteen of them fit easily into a teaspoon. (Courtesy of Dr. Carl G. Hartman.)

Marsupials

In the marsupials (of which only the opossum is found in this country), the yolk sac serves this function for a time. However, it does not become well-established, and as a consequence, the young are born in a very immature state (Fig. 21.9). They are able, though, to crawl into a special pouch on the mother's abdomen. There they attach themselves to her nipples and consume milk from her mammary glands until they are developed enough to fend for themselves.

The placenta

In all other mammals, the extra-embryonic membranes form a **placenta** and **umbilical cord** which connect the embryo to the mother's uterus in a more elaborate and efficient way. The blood supply of the embryo is continuous with that of the placenta. Although the capillaries of the placenta are in close contact with the mother's blood supply to the uterus, actual intermingling of the blood of the mother and the embryo does not normally occur. The placenta extracts not only food but also oxygen from the uterus. Carbon dioxide and other excretory wastes (e.g. urea) are similarly transferred to the mother's circulatory system for ultimate disposal by her excretory organs.

Although development continues within the uterus for a longer time than is characteristic of marsupials, there is a great variation among the placental mammals in the degree of helplessness of the newborn. In all cases, however, a period of feeding on milk supplied by the mother's mammary glands occurs. In some species, care of the young may be extended to include training in behavior patterns characteristic of the species, as illustrated by the red fox demonstrating to her cubs the techniques of hunting.

21.5 THE SEX ORGANS OF THE MALE

Man, of course, is a mammal. The gamete-producing organs, the gonads, arise early in embryonic development. In both sexes they arise low in the abdominal cavity. The gonads of the male are the two **testes.** At about the time of birth, they descend through the body wall into a sac, the scrotum. Occasionally, they fail to descend and the condition of cryptorchidism results. Unless corrected by surgery, no viable sperm are produced even after sexual maturity has been reached. The major difference between the location in the abdomen and the normal one is temperature. In the scrotum the testes are maintained at a temperature a few degrees below 98.6°F; this lower temperature is essential for the production of viable sperm.

Functions of the testes

Each testis consists of myriads of **seminiferous tubules** embedded in a matrix of **interstitial cells.** Lining the walls of the seminiferous tubules are the spermatogonia that produce the sperm. The interstitial cells produce the male sex hormones, the **androgens.** Androgens, of which testosterone is the most important, produce the secondary male sexual characteristics such as deep voice and facial hair. Sperm produced in the seminiferous tubules are collected by a series of tiny ducts, the *vasa efferentia,* which lead to a long (20-foot) coiled *epididymis* (Fig. 21.10). Here they are stored.

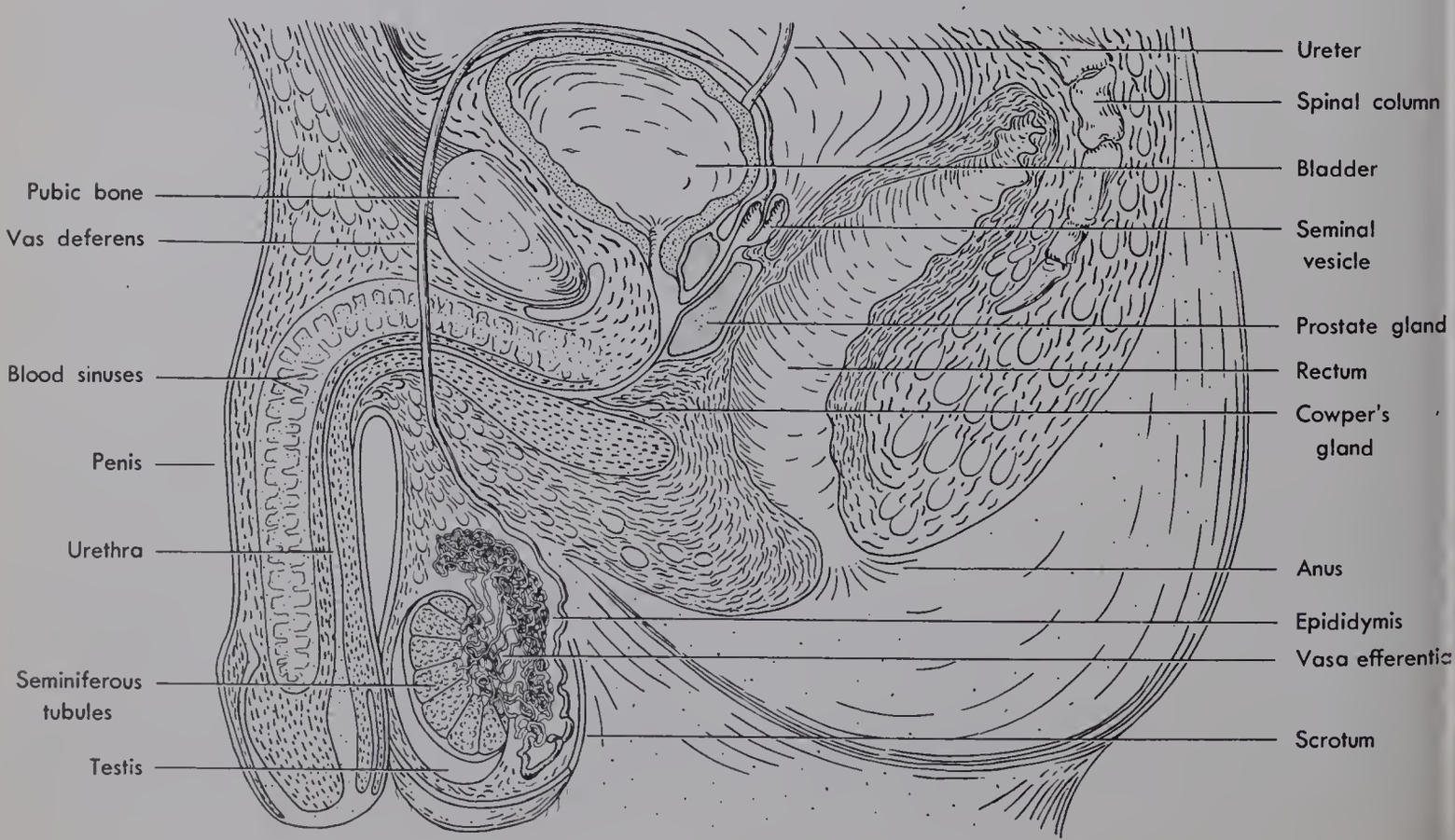


Fig. 21.10 Reproductory organs of the human male.

21.6 THE SEX ORGANS OF THE FEMALE

The gonads of the female are the two **ovaries** (Fig. 21.11). At the time of birth, hundreds of thousands of oogonia have started the first meiotic division to produce the same number of primary oocytes. No further development takes place until a girl reaches the period known as puberty (9–15 years). Then the eggs mature, usually one at a time and once a month. The egg grows much larger and completes its first meiotic division. This occurs within a **follicle,** a fluid-filled envelope of cells surrounding the egg. The follicle serves also as an endocrine gland. It produces a mixture of female sex hormones collectively known as **estrogens.** These are responsible for the development in the girl of the secondary sexual characteristics of a mature woman, such as broadening of the pelvis and development of the breasts.

Functions of the ovaries

The menstrual cycle

The estrogens produced by the follicle also play an important role in the monthly menstrual cycle of women. About once every 28 days (cycles somewhat longer or shorter or even irregular in length are found in perfectly healthy women), a small amount of blood and other products of tissue disintegration are discharged from the vagina. This process is called **menstruation.** It continues for four or five days during which time a new follicle begins to develop in one of the ovaries. After menstruation ceases, the follicle continues to develop, producing increasing amounts of estrogens as it does so. These stimulate an increase in the thickness and blood supply of the inner lining of the uterus. About two weeks after the onset

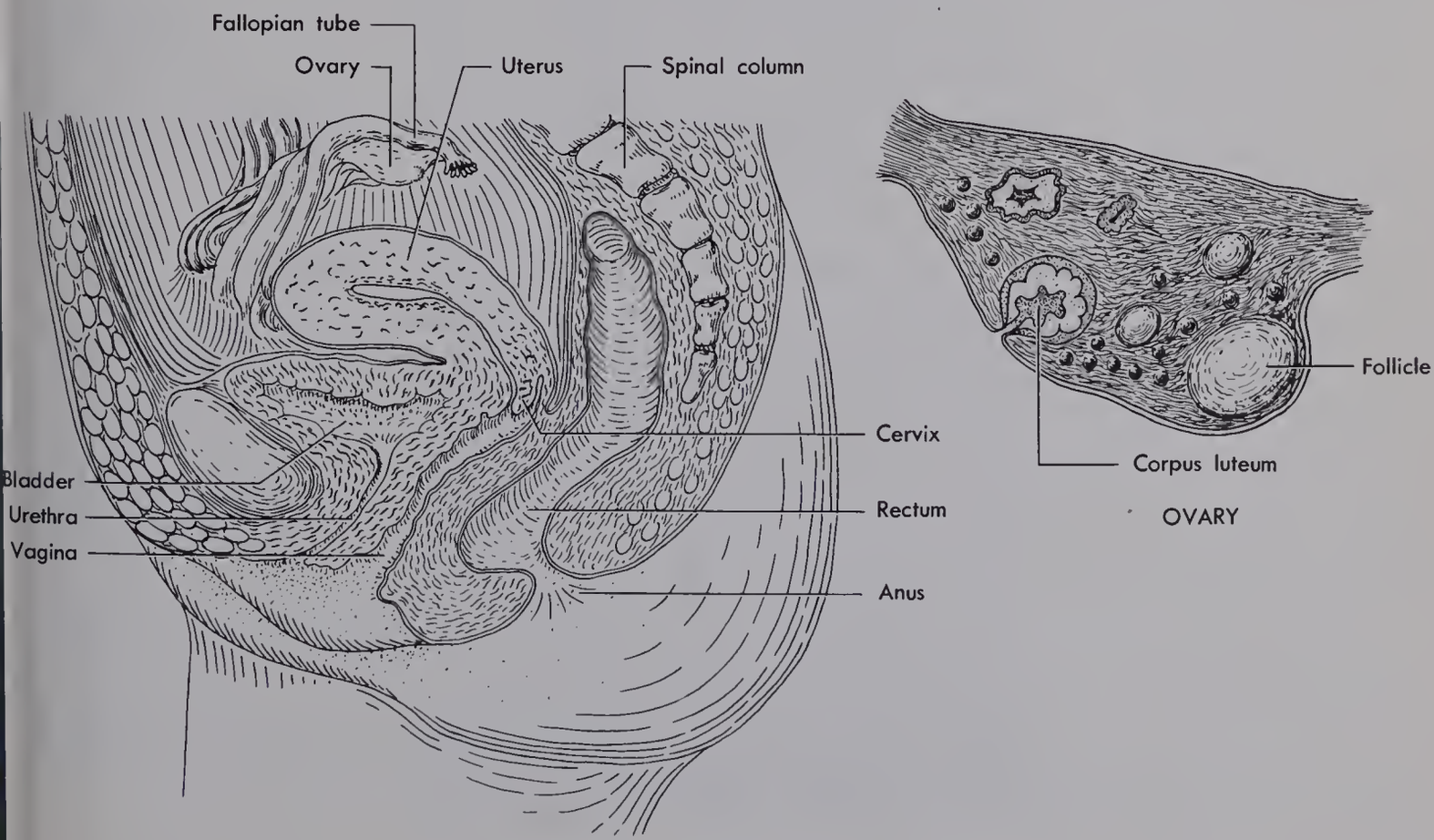


Fig. 21.11 Reproductory organs of the human female.

of menstruation, **ovulation** occurs. The follicle ruptures and discharges its egg. The egg is then swept into the open end of the oviduct (called a Fallopian tube in humans) and begins to migrate slowly down the length of the tube.

The old follicle undergoes a reorganization of its cells and develops into a yellow body, the **corpus luteum.** The corpus luteum also functions as an endocrine gland by secreting another female sex hormone called **progesterone** (Fig. 4.9). For about ten days, the high level of progesterone in the bloodstream exerts the following effects:

Functions of progesterone

1. It stimulates the further development of the lining of the uterus.
2. It inhibits contraction of the smooth muscle of the uterine wall.
3. It causes a slight enlargement of the mammary glands.
4. It inhibits the development of a new follicle.

If fertilization does not occur, the production of progesterone begins to drop off about the 26th day of the cycle. The corpus luteum shrinks, the lining of the uterus begins to break down, and by the 28th day, the menstrual flow begins again. Withdrawal of progesterone lifts the inhibition on uterine contraction. The "cramps" associated with the first day or two of menstruation result from vigorous contractions of the uterus. Withdrawal of progesterone also lifts the inhibition on follicle development so a new follicle begins to ripen and the cycle begins anew.

Menstruation in humans should not be confused with the periods of "heat" exhibited by many mammals, including the domestic dog and cat. These animals will allow copulation close to the time of ovulation only. In the process of ovulating the several eggs which a female dog may produce at one time, some blood is also produced. This flow is not related to that which occurs during menstruation.

21.7 FERTILIZATION, PREGNANCY, AND BIRTH

Copulation

For fertilization to occur, sperm must be deposited in the vagina fairly near to the time of ovulation. This is accomplished during copulation. As a result of sexual excitation, the arteries supplying blood to the penis of the male dilate. Blood accumulates in sinuses in the penis and the resulting pressure causes the penis to become erect and thus able to penetrate the vagina of the female. Further sexual excitation results in ejaculation. Sperm pass from the epididymis into the vas deferens. Peristaltic contractions of the smooth muscle of the wall of the vas deferens propel the sperm forward. Glandular secretions are added to the sperm by the seminal vesicles, the prostate gland, and the Cowper's glands (Fig. 21.10). These secretions probably serve to activate the sperm and to provide optimum chemical conditions for them in the female. The mixture of sperm and glandular secretions is called **semen.** The semen passes into the urethra and is expelled from the penis into the vagina.

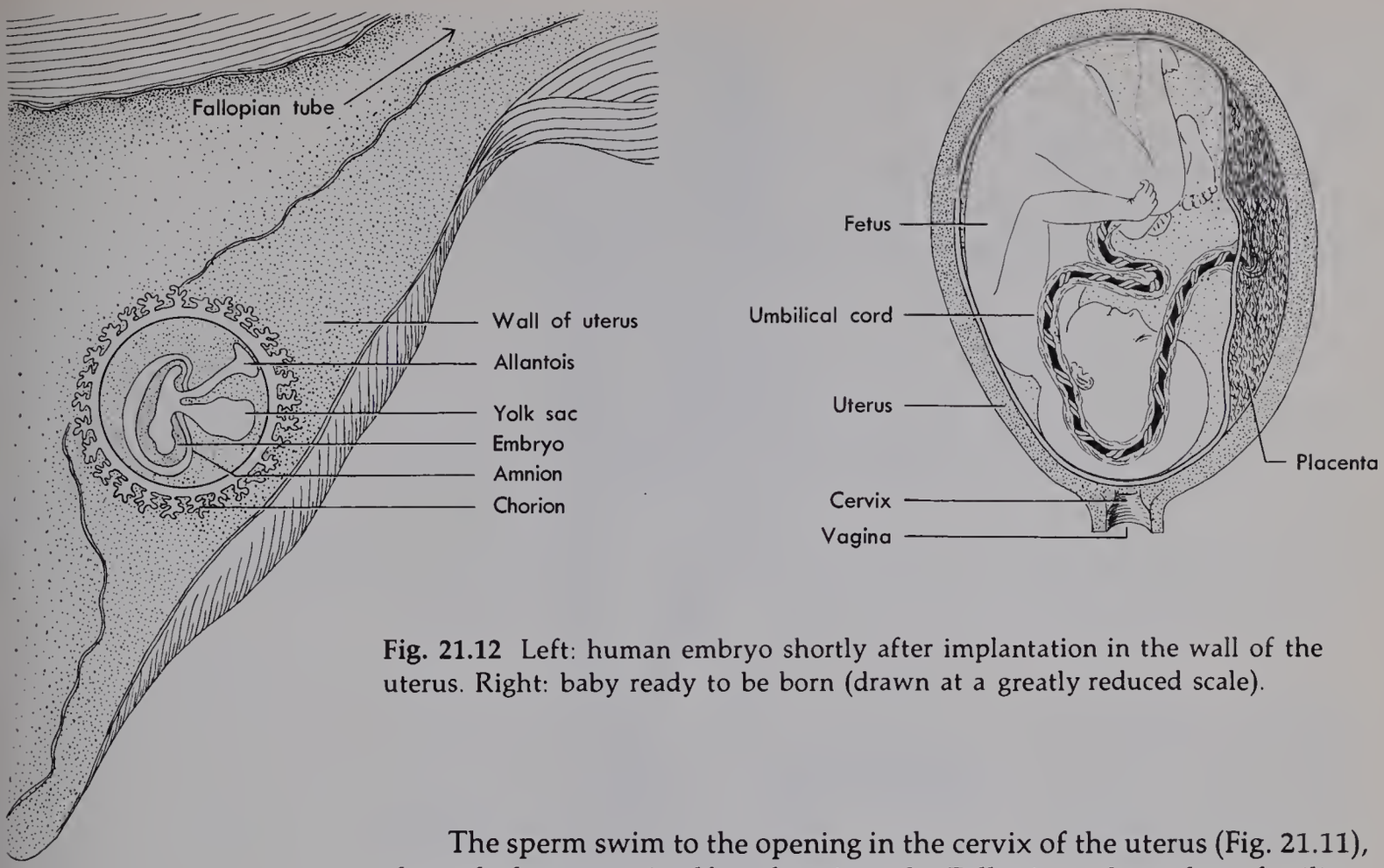


Fig. 21.12 Left: human embryo shortly after implantation in the wall of the uterus. Right: baby ready to be born (drawn at a greatly reduced scale).

Fertilization

The sperm swim to the opening in the cervix of the uterus (Fig. 21.11), through the uterus itself, and up into the Fallopian tubes where fertilization takes place if a viable egg is present. Although only one sperm penetrates the egg, medical authorities agree that at least 60 million sperm should be present in each milliliter of semen if fertilization is to occur. Most of the sperm die during the trip through the female genital tract.

Implantation

Embryonic development begins while the zygote is still within the Fallopian tube. The developing embryo continues to travel down the tube, reaching the uterus in about one week. Here implantation in the thickened lining of the uterus takes place. The extra-embryonic membranes develop and form the amniotic cavity, placenta, and umbilical cord (Fig. 21.12).

The placenta becomes an endocrine gland

With the implantation of the embryo, pregnancy is established. The corpus luteum continues to produce progesterone beyond the 26th day of the cycle and its effects are actually increased. But after five months, the corpus luteum is no longer able to produce sufficient progesterone to maintain pregnancy. By this time, however, the placenta itself has become active as an endocrine gland, producing supplementary amounts of progesterone. This placental progesterone violates the usual rule that hormones are transported in the blood. Instead, it simply diffuses from the placenta into the wall of the uterus where it serves to inhibit its contraction.

Relaxin

Exactly what brings about the onset of labor is still not fully understood. Probably a variety of carefully integrated hormonal controls are at work. Prior to the beginning of labor, the ovaries and placenta produce a hormone, called **relaxin,** which causes the ligaments between the pelvic bones to loosen. This provides a more flexible passageway for the baby

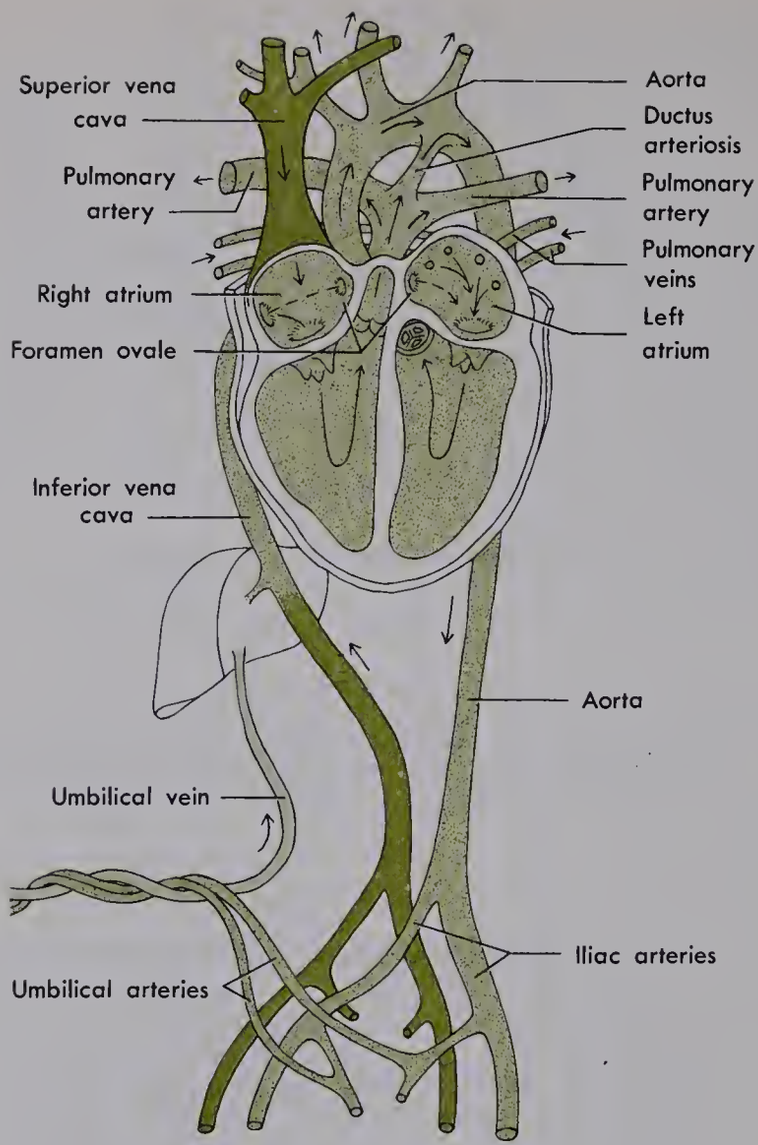


Fig. 21.13 Circulation in the human fetus. Oxygen is picked up at the placenta, not at the lungs. Most of the blood bypasses the lungs by passing through the foramen ovale and the ductus arteriosus.

during birth. Progesterone secretion by the placenta diminishes and this removes the inhibition that has kept the muscular wall of the uterus from contracting during pregnancy. Other hormones, which cause strong contractions of the uterus, make their appearance at this time. One of these

Oxytocin

is oxytocin, a hormone which will be discussed in Chapter 25. There is some evidence that it is the baby itself that is the chief source of oxytocin and, if so, we must give the baby credit for playing a major role in the onset

Prostaglandins

of labor. A group of closely related substances called prostaglandins also appear in the mother's blood and in the amniotic fluid at the time of birth. Prostaglandins, like oxytocin, cause the uterus to contract vigorously. These contractions produce the labor pains.

Birth

The cervix opens, the amniotic membrane ruptures and the amniotic fluid flows out. Finally, continued contractions of the uterus expel the baby through the cervix and out of the vagina. The umbilical cord can then be severed. The baby may have to be spanked to cause it to fill its lungs with air and commence breathing. Important changes also must occur in the baby's circulatory system so that a full supply of blood will be delivered to the newly inflated lungs (Fig. 21.13). Shortly after the arrival of the

baby, the placenta and remains of the umbilical cord (the "afterbirth") are also expelled.

Lactation

Within two or three days, the mammary glands of the mother begin to secrete milk. This is triggered by nervous stimulation provided by the baby's nursing and hormones produced by the pituitary gland, a tiny endocrine gland located at the base of the brain. (There are, in fact, a number of hormones produced by this gland which regulate the reproductory processes. Their precise role will be examined in Chapter 25.)

21.8 REPRODUCTIVE ENGINEERING: THE PROSPECTS

The rapid advances that have been made in the understanding of human reproductive physiology raise the prospect of manipulating the process in ways not hitherto possible.

Sperm banks

One technological development that is already upon us is the ability to freeze and store human semen for future use. Later (perhaps even after decades) the semen can be thawed and introduced into the reproductive tract of a woman. This procedure was originally developed as a convenient means of enabling prize bulls to inseminate cows anywhere around the world, thus quickly improving cattle breeds. Now the techniques are being used with humans. Several sperm banks have been created in the United States. Although thawed semen is not, as yet, quite as good as fresh semen (an average of 14 injections is needed to make a woman pregnant), the fact that the method works at all means that a man can now father a child after his own death (or after his sterilization, which appears to be the more immediate prospect). Furthermore, there is no technical reason why he could not become the father to great numbers of children born to many different mothers.

Embryo transfer

The transfer of a fertilized egg or early embryo from the female who produced it to a foster mother who will carry it to birth has not yet been achieved in humans but is done routinely with mice. In fact, early mouse embryos have been implanted successfully in foster mothers after being frozen and stored for over a week. R. G. Edwards and his colleagues at the University of Cambridge in England have succeeded in removing human eggs from their follicles (hence prior to ovulation), fertilizing them *in vitro* (in the "test tube"), and growing them to an embryonic stage known as the blastocyst. As yet, these have not been implanted in their "natural" or any other mother, but achieving successful reimplantation should be only a matter of time. The development of such a technique would permit mothers with closed Fallopian tubes to bear their own children. Furthermore, if a woman could not for some reason carry a baby to full term, reimplantation of the embryo in the uterus of a "foster mother" would still enable couples to have children that were genetically entirely their own.

The ability to maintain early embryos in culture for a short time would also make it possible to determine their sex before implantation. A few cells could be removed and checked for the presence of a *Y* chromosome or of a Barr body (see Section 18.3). In this way, only an embryo of

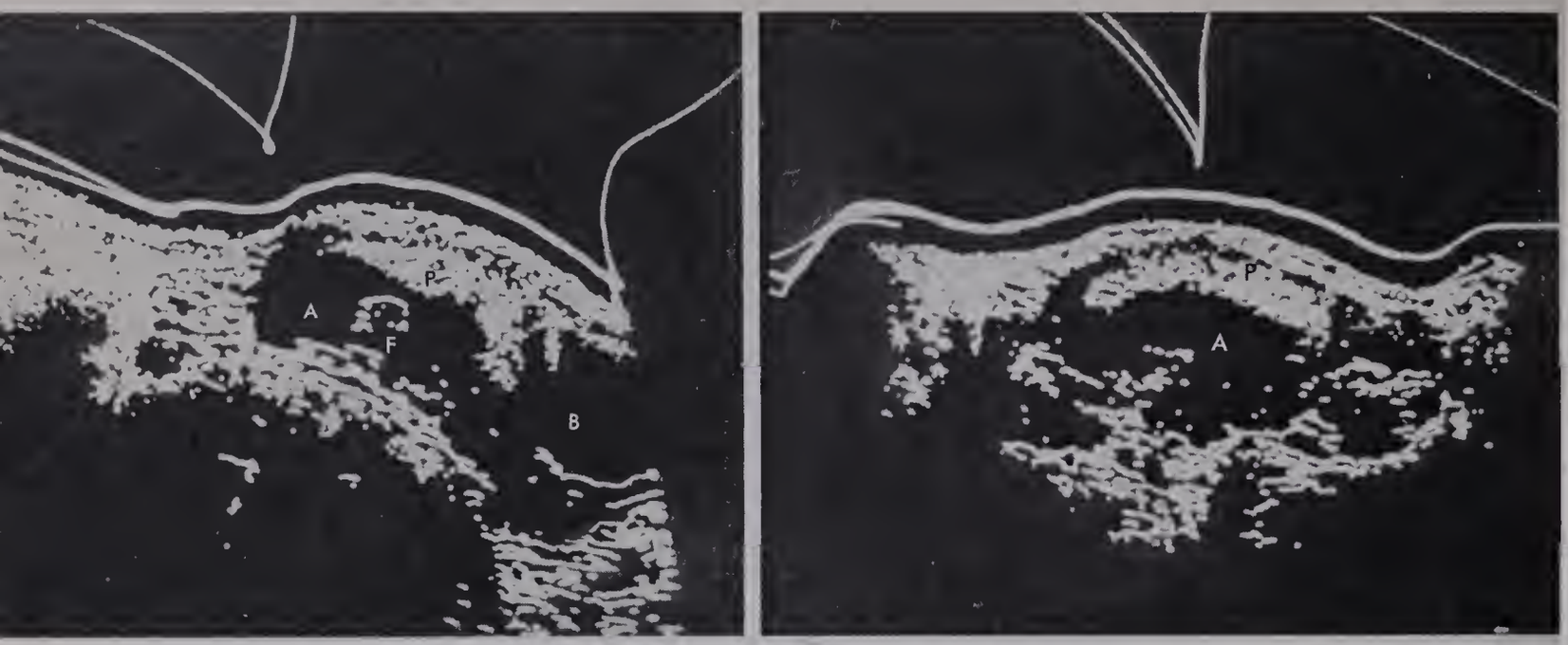


Fig. 21.14 Using ultrasound to locate the position of the placenta prior to amniocentesis. These sonograms are made by recording the echoes received from structures within the abdomen. A, amniotic cavity; B, urinary bladder; F, part of fetus; P, placenta. Both longitudinal (left) and transverse (right) scans are needed for accurate localization of the placenta. (Courtesy of the Downstate Medical Center of the State University of New York.)

the desired sex need be used. This technique has already been used successfully with rabbits. If (when) adapted to humans, it would permit, for example, carriers of severe *X*-linked traits, such as hemophilia, to elect not to run the risk of raising an affected son.

Amniocentesis

Actually, it is already possible to learn a great deal about the genetic makeup of the fetus. During its development, the fetus sheds cells into the amniotic fluid. Without too much difficulty, some of the amniotic fluid can be removed, a technique known as **amniocentesis** (Fig. 21.14). Separating the cells and culturing them enables the physician to look for chromosome abnormalities (e.g. the three number 21 chromosomes of Down's syndrome), certain enzymatic defects caused by point mutations (e.g. an inability to metabolize galactose, hence milk), and, of course, the sex of the embryo. Approximately 40 genetic disorders can now be diagnosed by amniocentesis, and the pregnancy deliberately terminated if a disorder is present.

Tetraparental mice

Not yet practicable for humans but by no means in the realm of science fiction is the production of children with *four* parents. For a number of years Beatrice Mintz and others have routinely produced such "tetraparental" mice. Early embryos at the eight-cell stage of development are removed from mother mice and placed in culture. If two embryos are pushed together, they often fuse into a single embryo. After a period of further growth, this fused embryo can be implanted in a foster mother and



Fig. 21.15 A tetraparental mouse. This mouse had two fathers and two mothers (not including the foster mother that gave birth to it). One pair of parents was black; the other pair was white. Its skin, as well as all its other organs, is made up of a mixture of cells from the black parents and cells from the white parents. Such an animal is called a genetic mosaic. Note that this mouse is not the same as an F_1 hybrid produced by mating a white mouse with a black mouse. In that case, all the cells would be of the same genotype (and the coat would have been a uniform brown). Photo (and mouse) courtesy of Dr. Thomas G. Wegmann.

go on to develop normally. The mouse that results is a mosaic (see Section 18.3) inasmuch as every organ is made up of some cells derived from one pair of parents and some cells derived from the other pair (Fig. 21.15). Such tetraparental mice have proven to be valuable tools for investigating a number of important biological problems. Whether tetraparental humans will be a blessing or a curse remains to be determined.

Contraceptive measures

Our discussion of methods of manipulating human reproduction should not ignore the most widespread techniques of all: those contraceptive measures by which couples block the start of pregnancy. These include mechanical barriers to the passage of sperm, the intrauterine device (IUD) which appears to prevent implantation, and the use of estrogen-progesterone preparations (the "pill") which block ovulation. (The function of these hormones will be considered further in Chapter 25.) Finally (and it is at present generally quite final), pregnancy can be avoided by tubal ligation or vasectomy. In tubal ligation, the Fallopian tubes are closed so that no egg can be fertilized. Ovarian function continues, however, including the normal hormonal cycle. In vasectomy, each vas deferens is cut near the top of the scrotum (Fig. 21.10) and tied. This is a minor surgical procedure that is performed in the doctor's office, under local anesthesia, in 30–40 minutes. The hormonal activity of the testes is not interrupted in any way. Nor does the operation stop the production of the various glandular secretions which make up the bulk of the semen. Hence copulation and ejaculation proceed perfectly normally.

Whatever the technological methods employed, reproductive engineering constitutes a special case of genetic engineering (see Section 19.10) and raises the same ethical problems in addition to some special ones of its own. It is to be hoped that the associated ethical and legal questions will be dealt with before the need to do so becomes critical.

EXERCISES AND PROBLEMS

1. The retention of fertilized eggs within the mother's body occurs commonly among the cartilaginous fishes and the reptiles. Why do you suppose no birds employ this means of protecting their eggs?
2. Distinguish between sperm and semen.
3. In what ways is egg formation in the frog similar to megaspore formation in the bean? In what ways is it different?
4. What is the difference between amplexus and copulation?
5. Where does fertilization occur in (a) the trout, (b) the shark, (c) the queen bee, (d) the frog, and (e) the human?
6. If a family has had seven daughters and no sons, what are the chances that their next child will be a son?
7. Neglecting crossing over, how many kinds of sperm cells can be produced by *Ascaris bivalens* (n = 2)? How many kinds of egg cells can be produced?
8. Few animals are hermaphroditic, while most plants are. Can you think of an explanation for this?
9. The number of eggs produced by the members of a species can be related to the amount of care given to the developing young. Is the same true of the number of sperm produced? Explain.
10. What functions do the centrioles of the spermatogonia carry out (a) in sperm formation, (b) in sperm transfer, (c) in fertilization?

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22

DEVELOPMENT: CLEAVAGE, MORPHOGENESIS, AND DIFFERENTIATION

Stages in the Development of the Adult

Cleavage

Morphogenesis

Differentiation

What Controls Development?

The Genes—a Problem

Cytoplasmic Factors

Extracellular Factors

Selective Translation of the Genetic Code

The Reversibility of Differentiation

22.1 STAGES IN THE DEVELOPMENT OF THE ADULT

In the past two chapters we have examined the ways in which gametes are manufactured and brought together so that fertilization can occur. Fertilization is not the end of the process of sexual reproduction. It is, in fact, the start of a series of elaborate and well-organized changes which eventually give rise to a new adult of the species. We use the term *development* to describe these changes. Although the exact pattern of development varies from one species to another (especially if they are not closely related) and there is some overlapping, one can usually distinguish the following stages.

1. **Cleavage.** During this stage of development, the zygote nucleus undergoes a series of mitotic divisions. The resulting daughter nuclei usually become partitioned off in separate cells fashioned from the cytoplasm of the zygote. There is little or no growth during this stage.

Extensive cell migration occurs during morphogenesis in animals

2. **Morphogenesis.** During this stage, the many cells produced by cleavage continue dividing but also move about and organize themselves into distinct layers and masses. As a result, a definite pattern appears. It is this development of pattern that is called morphogenesis. Although the cells of the embryo during this phase of development are organized into distinct groups, they are all rather similar in structure.

Differentiation is the acquisition of specialized structure and functions

3. **Differentiation.** Soon, however, the cells of the developing embryo begin to take on the specialized structure and functions which they will have in the adult. Nerve cells, muscle cells, etc., are formed. This process is called differentiation. The differentiated cells are organized into tissues, the tissues into organs, and the organs into systems.

4. **Growth.** Even after all the body systems of the organism have been formed, there follows a period of growth. During this period, the organism becomes larger by continued cell division or by cell enlargement, or both. Whatever the exact mechanism, growth depends on the intake of more matter and energy than is needed simply to maintain the normal functions of the organism. There must be an accumulation of proteins, carbohydrates (especially in plants) and the many other kinds of molecules characteristic of living things. While an organism is passing through the first three of these developmental stages, it is called an **embryo.** Let us now examine these stages in more detail.

22.2 CLEAVAGE

As we saw in the last chapter, the entrance of a frog sperm into the frog egg initiates several events. The "gray crescent" appears, meiosis of the egg is completed, and then the sperm nucleus and egg nucleus fuse. Shortly after this vital part of the fertilization process is completed, the first cleavage takes place. The zygote nucleus divides by mitosis, and a furrow appears which runs longitudinally through the "poles" of the egg (Fig. 22.1). This soon divides the egg into two halves.

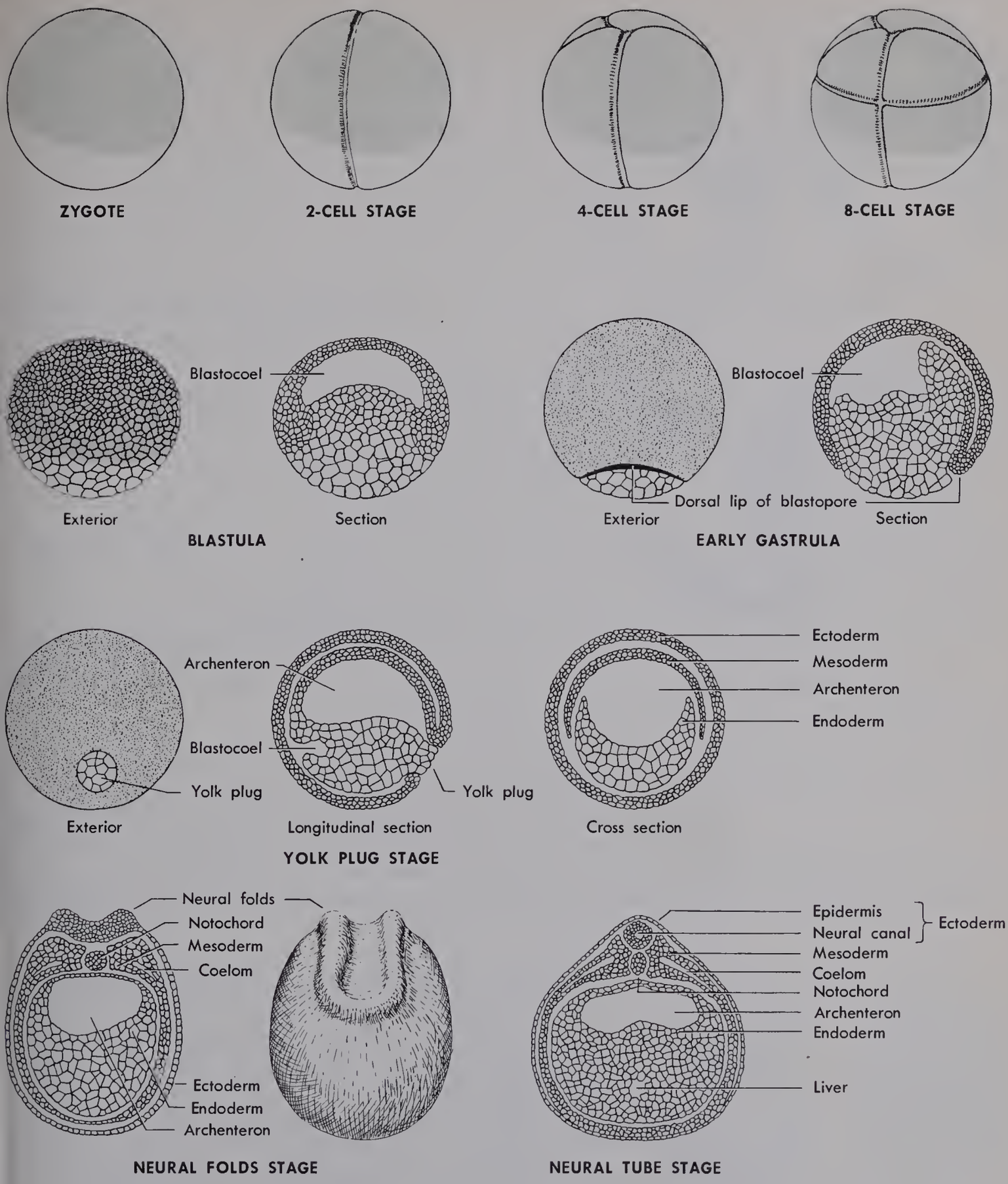
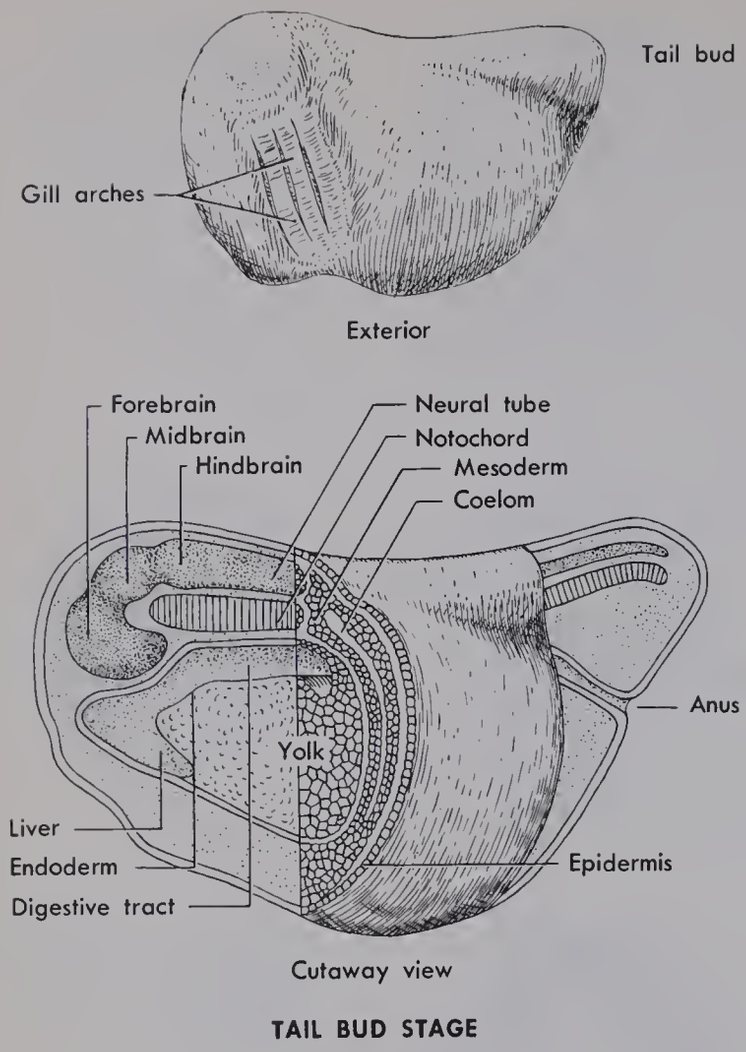


Fig. 22.1 The stages in the development of a frog. (cont. on next page)

Fig. 22.1 (cont.)



About an hour after the first cleavage in the frog egg, each of the daughter cells divides again. The resulting cleavage furrow also runs through the poles but at right angles to the first. Each of the four resulting cells then simultaneously divides in a horizontal plane (Fig. 22.1). This plane is located closer to the animal pole than the vegetal pole. As a result, the cells of the animal pole are somewhat smaller than the yolk-filled cells of the vegetal pole. Simultaneous cleavage continues with the production of a 16-cell and then a 32-cell stage.

The blastula

As cleavage continues, the animal pole cells begin to divide more rapidly than those of the vegetal pole. The former thus become not only smaller but also more numerous. During this period some migration of the cells of the animal pole takes place. These orient themselves so that a fluid-filled cavity, the **blastocoel,** forms inside the mass of cells. This hollow-ball-of-cells stage is called the **blastula.** It marks the end of the cleavage stage of development. Note that during this entire stage there has been no growth of the developing organism. The original mass of the egg has simply been partitioned into smaller and smaller units.

Cleavage of the eggs of reptiles and birds

Reptiles and birds produce the largest eggs of all living things. For example, the hen's egg consists of just a tiny patch of cytoplasm resting on the surface of a large ball of yolk. (The "white" of the egg is simply

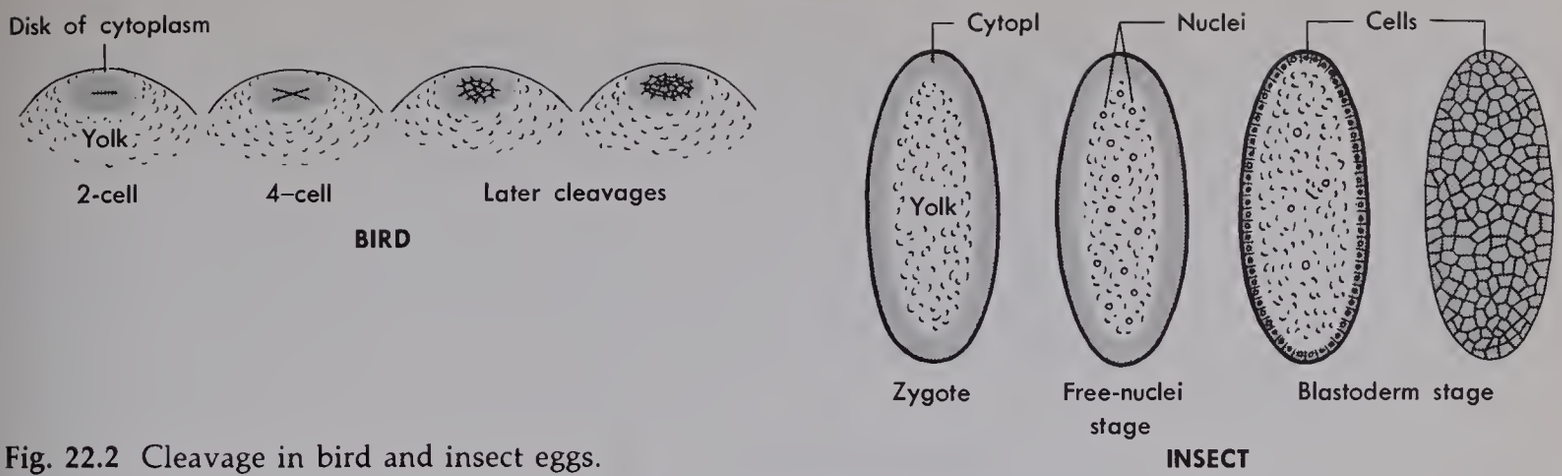


Fig. 22.2 Cleavage in bird and insect eggs.

noncellular accessory protein.) When cleavage occurs in the hen's egg, the cleavage furrows do not continue down through the mass of yolk. As a result, each of the cells produced in the early cleavage stages is bounded on the top and on the sides by a cell membrane, but the bottom of the cell is in direct contact with the mass of yolk (Fig. 22.2).

Cleavage of the insect egg

The yolk of insect eggs is concentrated in the center of the egg. Cleavage of the egg does not accompany mitosis of the zygote nucleus. Instead, the daughter nuclei divide repeatedly but remain suspended within the single egg compartment (Fig. 22.2). After a large number of nuclei have been produced, they migrate to the cytoplasm-rich margin of the egg. Only then do cell membranes form around each one.

Early development in plants

Development of the zygote in plants proceeds somewhat differently than in animals. Mitosis of the zygote is usually accompanied by cell enlargement. In the gymnosperms and angiosperms, some of the daughter cells elongate very rapidly. Their growth pushes the remaining cells up into the food-rich endosperm. The endosperm provides the materials for further development of the embryo (Fig. 22.3).

Functions of cleavage

What does cleavage accomplish in the development of the organism? First, it provides a stockpile of cells out of which the embryo can be constructed. We have seen that cells are the structural unit of almost all

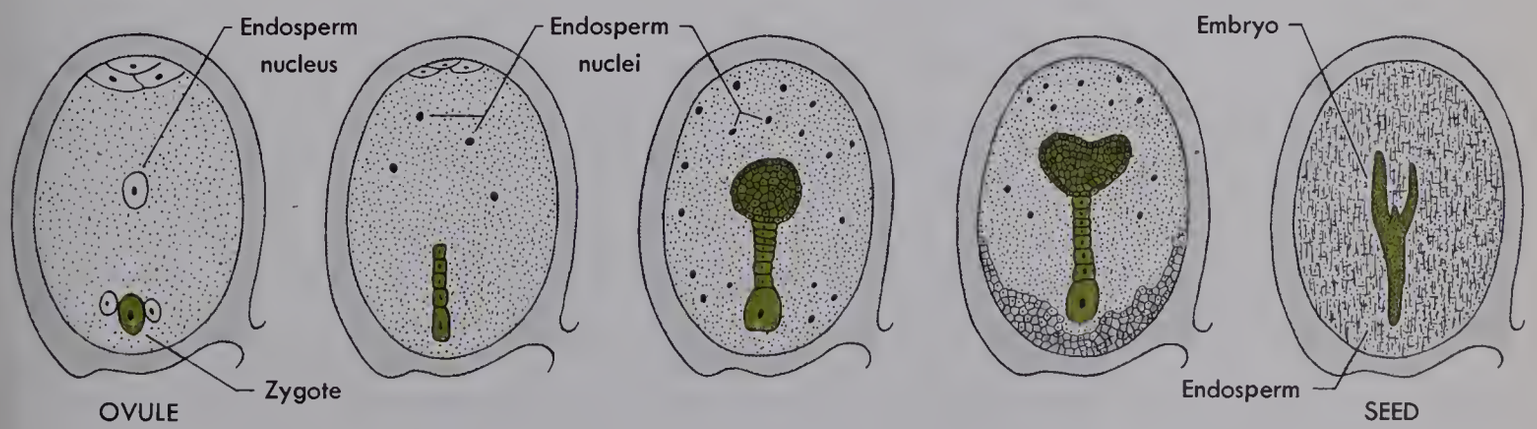


Fig. 22.3 Cleavage and morphogenesis in the angiosperm. Nourishment for the developing embryo is secured from the endosperm. The scale of the drawings decreases from left to right.

living things. Cleavage establishes a supply of them. Second, cleavage establishes a normal relationship between the nucleus and the cytoplasm it regulates. Even small eggs are enormous when compared with other kinds of cells. The volume of the frog egg is about 1.6 million times larger than that of a normal frog cell. But it, too, contains only one nucleus. During the process of cleavage, thousands of new nuclei are produced by mitosis. Each of these eventually becomes established in a cell of normal dimensions. Remember that the frog blastula with its thousands of cells is no larger than the original zygote.

22.3 MORPHOGENESIS

Cell division does not cease with the blastula stage. In the frog the smaller, darker cells of the animal pole continue to undergo rapid mitosis. As the number of these cells increases, they begin to migrate down over the yolk-filled cells of the vegetal pole. Some of the vegetal pole cells also begin to migrate. In their case, they push into the interior of the cell mass, producing a small crescent-shaped depression known as the blastopore (Fig. 22.1). This pushing-in of cells marks the start of **gastrulation.** As the animal-pole cells migrate down over the surface of the cell mass, a large number of them also push into the interior at the region of the blastopore.

Gastrulation

The inpocketed vegetal pole cells develop into a sheet of cells which grows upward and eventually encloses a cavity, the archenteron (Fig. 22.1). These cells are known as **endodermal** cells. From the archenteron will develop the alimentary canal, the liver and other digestive glands and, after metamorphosis, the lungs. At this stage, the only opening to the archenteron is at the blastopore. Ultimately, the anus will form here. The inner lining of all these organs will be made of endodermal cells directly descended from the first vegetal-pole cells.

Endoderm

The animal-pole cells growing down over the embryo also migrate inward. This occurs first at the upper (or dorsal) portion of the blastopore. The cells move in directly beneath the overlying animal-pole cells. They are now called **mesodermal** cells. From them develops a rodlike structure, the **notochord,** which runs lengthwise along the dorsal side of the developing embryo.

Mesoderm

As gastrulation continues, animal-pole cells crowd in around the entire margin of the blastopore. These late arrivals also become mesoderm. Those nearest the notochord become organized into separate masses called **somites,** from which will develop muscles and the bones of the vertebral column. Nearer the ventral surface of the embryo, the mesodermal cells develop into the lining of the **coelom,** the main body cavity. The cavity itself arises by a splitting of this mesoderm (Fig. 22.1). This stage of development is often known as the yolk-plug stage because only a small disk of yolk cells is still visible at the exterior. The animal-pole cells that do not migrate into the interior are called **ectodermal** cells. From them will arise all the structures of the nervous system as well as the skin of the animal.

Ectoderm

Shortly after the formation of the notochord, the ectodermal cells immediately above it undergo a period of rapid cell division. This results in a deep, flat layer of cells called the neural plate (Fig. 22.1). Then the edges of the neural plate grow up while the central region just above the notochord becomes depressed. This so-called neural-folds stage is followed by the formation of a neural tube. The two elevated folds grow over and fuse together along their entire length. From this structure develops the brain and spinal cord. In Fig. 22.1 you can see that three enlargements of the neural tube form at the anterior end of the embryo. These will develop into the brain. The neural tube then drops down into the embryo and is covered over by another layer of ectodermal cells which develops into skin.

Although the details of gastrulation vary considerably from species to species, the variations are superficial rather than fundamental. In every case, the process results in the formation of three primary cell layers (often called "germ" layers), the **ectoderm, mesoderm,** and **endoderm.** From these three germ layers arise the various organs and systems of the animal body. Figure 22.4 lists the germ-layer origin of the various organs and systems in man. This list could serve as well for any other vertebrate.

ECTODERM
- Skin, hair, nails
- Entire nervous system, including receptor cells
- Adrenal medulla

MESODERM
- Muscles
- Blood and blood vessels
- Connective tissue, including bone
- Kidneys, ureters
- Testes, ovaries, oviducts, uterus
- Mesenteries
- Lymphatic system

ENDODERM
- Lining of alimentary canal
- Lining of trachea, bronchi, and lungs
- Lining of urethra and bladder
- Liver
- Pancreas

Fig. 22.4 Germ-layer origin of the various body tissues.

The process of gastrulation establishes the basic pattern of the embryo. Cells are stockpiled in specific locations in preparation for the construction of the various tissues, organs, and systems of the organism. Their construction involves more than simply the migration and stockpiling of cells in the three germ layers, however. The unspecialized cells of ectoderm, mesoderm, and endoderm must all take on specialized shapes and internal organization to permit them to carry out the functions of the tissue or organ in which they are located. This conversion of unspecialized cells into specialized ones is called differentiation.

22.4 DIFFERENTIATION

Although the later stages of morphogenesis and the process of cell differentiation overlap to a considerable extent, it is well to keep in mind the distinction between the two processes. Morphogenesis involves the organization of cells into the various layers and groups that will form the structures of the body. It involves cell division and the actual movement of cells from place to place within the embryo. Although the various layers of cells in the frog gastrula have definite and different fates ahead of them, they do not at first reveal these fates by any specialization of structure or function. In fact, it is possible for a careful experimenter to transplant cells from one location to another in the early gastrula; these transplanted cells readily adapt to their new location and go on to participate in the building of an organ appropriate to it.

As embryonic development proceeds, however, the cells of the developing embryo reach a "point of no return." They become committed to the formation of a specific kind of cell. Gradually they take on the appearance

Differentiated cells specialize in the synthesis of one or a few characteristic proteins

and function of the various kinds of cells discussed in Chapter 5. As they do this, each begins to synthesize a small number of proteins characteristic of that cell type and no others. Differentiating heart muscle cells start synthesizing a special contractile protein called heart myosin. Differentiating red blood cells begin synthesizing hemoglobin. As connective tissue cells differentiate, they become committed to the synthesis of collagen and other extracellular proteins. And so on. This commitment to a specific fate seems to occur first in mesodermal and endodermal cells, then in the ectodermal cells.

Although the processes of cleavage, morphogenesis, and cellular differentiation can be distinguished from one another, the three operations share one fundamental similarity. In each case, unlike things are being made from like things. From the eight-cell stage of frog cleavage, the cells of the animal pole differ in appearance and chemistry from those of the vegetal pole. From the hollow sphere of the blastula arise three distinct germ layers. From the unspecialized cells of the three germ layers are formed all the differentiated cells of the mature organism. Furthermore, the development of all these diverse structures does not proceed in a random, haphazard way. The precise arrangement of the cleavage planes, the migration of cells in gastrulation, and the production of just the right kinds and numbers of cells in the process of cellular differentiation all reveal a wonderful degree of orderliness and organization. Indeed, at every stage of embryonic development it seems as though the operations are being carried out under the guidance of some master plan.

WHAT CONTROLS DEVELOPMENT?

What is the nature of this guiding plan? Although research workers have tried for years to discover the answer to this question, we are only just beginning to glimpse the mechanism by which the zygote gives rise to the adult. Of one thing we may be quite sure, however: the master plan of development must reside within the egg or sperm or both. When we consider the large number of organisms (including the frog) that develop far from any adult body, this conclusion seems inescapable. The only essential contribution that adults make to the formation of the next generation is the gametes—the egg and sperm.

22.5 THE GENES—A PROBLEM

Boveri's work with haploid sea urchins (see Section 5.4) suggests strongly that it is the nucleus which contains the master plan or blueprint for the development of the organism. After all, the sperm itself is little more than a flagellated nucleus. Within the nucleus are the chromosomes and, as we saw in Chapter 18, the evidence is overwhelming that they are the bearers

of the genes. Genes control the traits of an organism, that is, its phenotype. But the phenotype is itself simply the ultimate expression of a long series of developmental stages. Assuming, then, that it *is* the genes that control the development of an organism, a crucial question still remains unanswered.

Every cell contains the same complement of genes

So far as we can tell, all the trillions of differentiated cells in the adult human have developed from the zygote by mitosis. If this is true, they all contain the same chromosomes that the zygote contained: no more, no less. This suggests that every cell contains *all* the genes for constructing an entire organism. How, then, can we explain the fact that during the course of embryonic development certain cells migrate and differentiate in one way while other cells develop quite differently? How can we explain the diverse fates of a trillion cells each of which contains the same genes that the zygote contained?

22.6 CYTOPLASMIC FACTORS

Many of the tentative answers we now have to this question have come about as a result of the work of experimental embryologists. These are biologists who, by a variety of techniques, alter the path of normal embryonic development. By observing what happens under abnormal conditions, they gain clues as to the mechanisms that operate under normal ones.

One of the earliest experiments of this sort involved the physical separation of the cells produced in the early stages of cleavage. It is known that each of the two cells produced when the frog zygote undergoes its first cleavage will normally give rise to one-half the embryo. One might expect that in some way each of these two cells is able to use only one-half of the genes of the organism. Physical separation of the cells shows, however, that such is not the case. Reared separately, each goes on to produce a complete, although smaller, embryo.

Monozygotic ("identical") twins

This discovery provides an explanation for the occurrence of identical twins, those twins that show such a striking resemblance to each other. Identical twins are always of the same sex and even in their body chemistry seem to be identical in every way. We explain these rare individuals by assuming that they came from a single zygote which, after one or two cleavages, was separated into two parts. Identical triplets, quadruplets, and quintuplets undoubtedly arise in a similar fashion. The remarkable similarity of these individuals is understandable when we realize that they were formed from a single zygote and thus a single set of genes.

When the cell-separation experiment is repeated at the four-cell stage of cleavage, the results are quite different. None of the resulting cells, when isolated, can produce a complete embryo. What has happened? Have the daughter nuclei produced during the second cleavage lost some of the genes necessary to construct the total organism? The answer to this question was discovered by the German experimental embryologist, Hans Spemann. Using fine strands of baby hair, he tied tight loops around fertilized newt eggs (which are similar to frog eggs) so that they were constricted

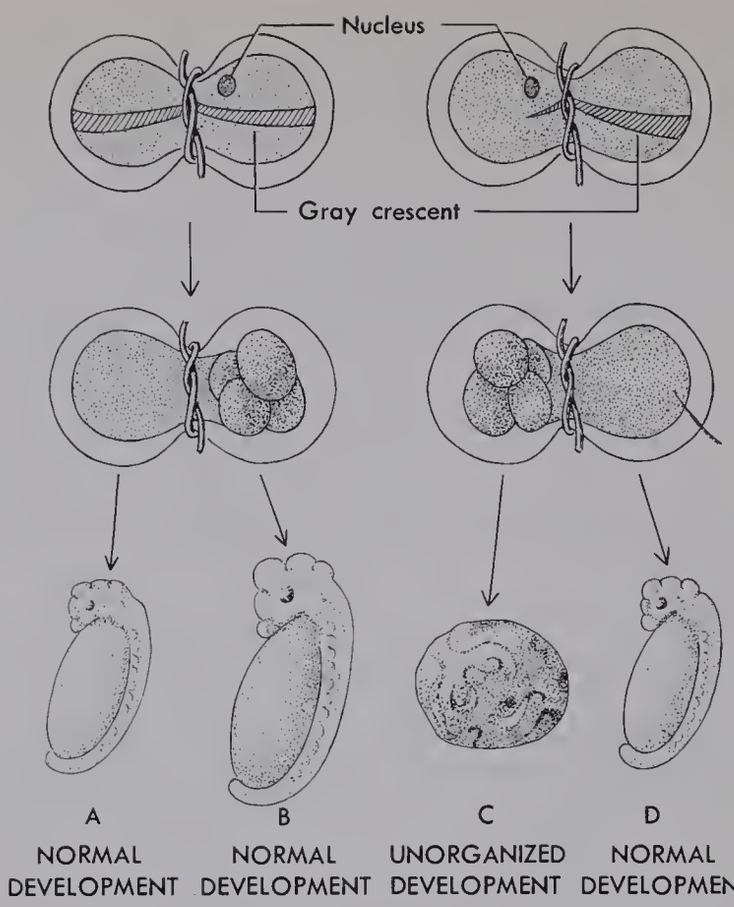


Fig. 22.5 Spemann's experiment. Even though Embryo C started development earlier than Embryo D, it never developed beyond an unorganized mass of belly tissues.

Spemann's experiments

longitudinally into two halves (Fig. 22.5). The zygote nucleus was in one half. The other half had no nucleus but was in contact with its nucleus-containing partner by a narrow bridge of cytoplasm. Spemann found that only the half-egg with the nucleus in it would cleave. However, at some point during cleavage, a nucleus usually did get across the narrow connecting bridge of cytoplasm. As soon as this happened, the second half began to cleave, too. Even though its nucleus was the product of as many as five prior cleavages, it went on to form a complete embryo. Clearly, then, the nucleus, at the 32-cell stage at least, has not lost any of the genes for making the organism.

When Spemann repeated his experiment with the fertilized egg constricted so that all of the gray crescent lay in one half, the final results were quite different (Fig. 22.5). The half lacking the gray crescent but containing the nucleus began cleavage right away but never developed beyond an unorganized mass of intestinal, liver, and other abdominal cells. The other half, even though it did not get a nucleus until the fourth mitotic division on the cleaving side, went on to form a perfectly normal embryo. This again was proof that the nuclei, here at the 16-cell stage, had not lost any genes. But why, then, did a normal embryo fail to develop on the side with the original zygote nucleus?

We have already seen that the distribution of cytoplasm, mitochondria, RNA, ribosomes, and yolk in the amphibian egg is not uniform. Shortly after fertilization, some of the cytoplasmic constituents migrate and form the gray crescent. Perhaps the potentialities a nucleus can achieve

The importance of the gray crescent

are regulated by the cytoplasmic environment in which it finds itself. Thus, in Spemann's first experiment, each half of the egg contained all the normal egg constituents, because the longitudinal constriction was perpendicular to the gray crescent. However, in his second experiment, the hemisphere lacking the gray crescent may have lacked some essential cytoplasmic materials.

The importance of the cytoplasmic environment may explain the results of the cell-separation experiments described earlier. When the first cleavage in the frog egg occurs perpendicular to the gray crescent (as it often does), each of the two cells produced contains all of the normal egg constituents. The second cleavage, however, isolates the gray crescent in two of the four cells produced. Although all the cells contain nuclei with the complete hereditary blueprint, we may assume that the action of the genes is now limited by the cytoplasmic surroundings in which they find themselves. Though the nuclei are all alike, the nucleus-cytoplasm combinations are not, and we have our first clue as to the way in which dissimilar cells can arise from a single zygote.

22.7 EXTRACELLULAR FACTORS

The experiments we have been describing show that an unequal distribution of substances within the zygote gives rise to cells of diverse fates despite their identical nuclear controls. It is difficult to imagine, though, that the relatively simple gradients involved account for all the complex migration and differentiation of cells during embryonic development. Are there other guiding forces at work during embryonic development?

To Hans Spemann must also go the credit for discovering that there are indeed other guiding forces. You remember he had found that half a newt zygote would develop normally so long as it (1) contained the nucleus and (2) contained some of the region of the cytoplasm known as the gray crescent. Spemann knew that it is the cells that develop in the region of the gray crescent which migrate through the blastopore during gastrulation and form the notochord. With great surgical skill, he succeeded in cutting out the cells of the dorsal lip of the blastopore before their inward migration (Fig. 22.6). Then he transplanted this tiny bit of tissue into the ventral

Transplanting the dorsal lip of the blastopore

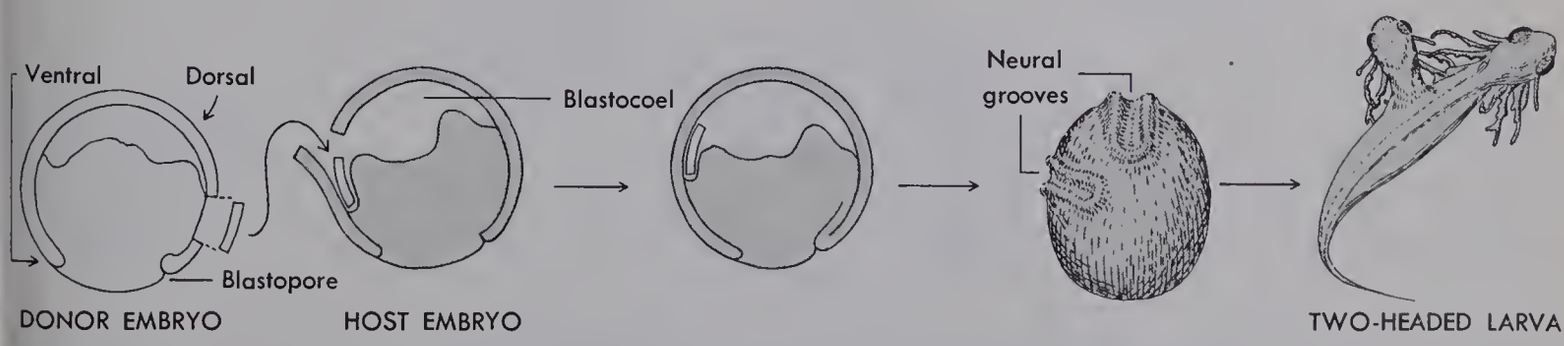


Fig. 22.6 Another of Spemann's experiments. Donor tissue (white) from the region of the gray crescent developed into a notochord and induced the formation of a second head in its host (gray).

side of a normal newt gastrula. To make it easier for him to follow the fate of the transplant, he used the early gastrula of a pale variety of newt as the donor and the early gastrula of a dark variety as the recipient or host. He discovered that the transplanted piece of dorsal lip developed into a second notochord. Above the site of the transplant a neural groove appeared. This neural groove was made up almost exclusively of host (dark) cells. It underwent normal development, forming a neural tube and then a central nervous system. Ultimately a two-headed monster was produced.

The fact that most of the tissues of the second head were derived not from the transplanted cells, but from the host cells, suggested to Spemann that the transplanted cells had altered the normal course of development of the host cells around them. Instead of producing the belly of the newt, the host cells above the transplant were stimulated to produce a second central nervous system. We use the term **induction** for this process.

Induction

When Spemann transplanted portions of gastrulas other than the cells of the dorsal lip of the blastopore, no induction was observed. The transplanted tissue simply developed according to its new location. Because only the dorsal lip of the blastopore could induce altered development in the host, Spemann called it the *organizer*. He visualized the organizer as initiating the process of morphogenesis and differentiation by inducing the formation of the neural tube. The neural tube then might induce the formation of still other embryonic structures. For example, we know that as the brain develops, two masses of nervous tissue, the optic cups, grow forward from it. As these near the anterior surface of the embryo, skin cells just in front of them differentiate to form lenses (Fig. 22.7). The optic cups become the retinas of the finished products, the eyes.

The "organizer"

The theory of induction explains very nicely this transformation of skin cells into lens cells. In fact, it is not hard to visualize the development of the entire embryo by this mechanism. As each structure is induced, it

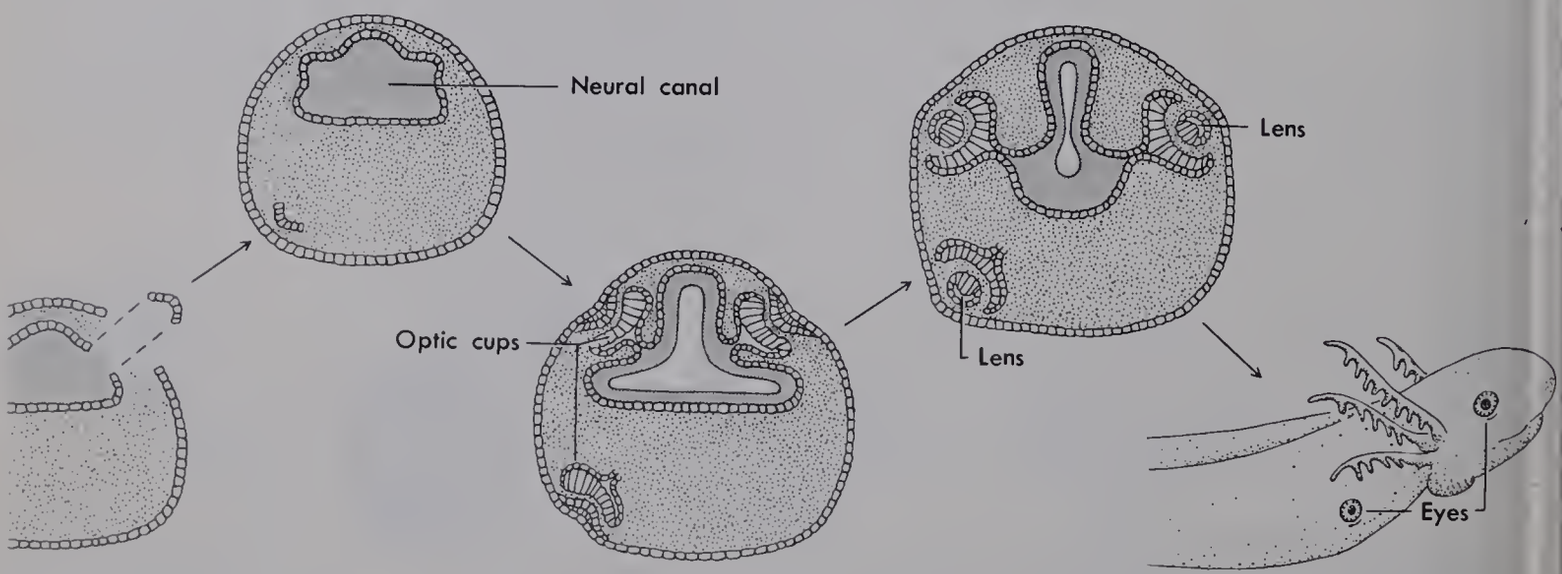


Fig. 22.7 Embryonic development of the salamander eye. A transplanted optic cup induces lens formation in the tissues of its host and an extra eye develops.

can then induce still other structures. Successive waves of induction could thus account for the complete, organized embryonic development of the animal.

Not long after Spemann's discovery, it was found that the inducing properties of the organizer were retained even if the cells were killed. This immediately suggested that induction is accomplished by the passage of some chemical substance from the organizer to the affected cells. It was thought that the inducing substance instructed the affected cells to organize and differentiate in a specific way. This idea thus provided another plausible answer to our question of how diverse cells arise from a single zygote. The peculiar cytoplasmic composition of the gray crescent region might stimulate the blastula cells produced in that region to liberate special chemical regulators. These cells would thus have a feature unique in the embryo and could become the organizer. The diffusion of chemical substances from them might then instruct adjacent cells to develop in a special way. As the latter developed, they in turn could release inducing substances which would program the differentiation of still other cells. In this way the entire embryo could be built.

With such an attractive theory, it was natural that every effort be made to isolate and identify inducing substances, not only in amphibian embryos but in other organisms as well. In a number of cases it was found that induction could occur across a gap filled with agar-agar, but not across a sheet of cellophane. This suggested that a fairly large molecule was involved and, indeed, induction has in some cases been accomplished *in vitro* by treating the tissues with a relatively pure protein preparation. Some workers have also presented evidence that ribonucleoprotein, i.e. a complex of RNA and protein, may have inductive properties.

Evidence that inducers are macromolecules

Some small molecules act as inducers

Although most inducers appear to be macromolecules, there are some exceptions. Vitamin A, a small molecule, can induce altered development in the epidermal cells of a chick embryo. Other small molecules such as certain steroids and even the dye methylene blue (which is not a normal constituent of cells) can induce neural plate formation in the amphibian embryo. The inductive changes produced by these small molecules are not as elaborate as those produced by proteins. Methylene blue simply shifts the development of epidermis to neural tube, both of which are ectodermal structures. Induction by macromolecules can, on the other hand, convert cells that would have developed into ectodermal structures into mesodermal tissues, like muscle, and even into endodermal tissues.

Evidence of inhibitory controls in development

The differentiation of cells seems also to be influenced by *inhibitory* substances that reach them from adjacent cells. When developing frog embryos are placed in cultures containing pieces of adult frog heart, the embryos fail to produce a normal heart. Similarly, embryos cultured with pieces of adult brain fail to produce a normal brain. This would suggest that differentiated cells can produce substances that inhibit adjacent cells from differentiating the same way. Certainly in adult organisms this is the case. Differentiated tissues secrete substances (proteins and/or polypeptides) called **chalones** which inhibit mitosis of the cells of that tissue.

Chalones

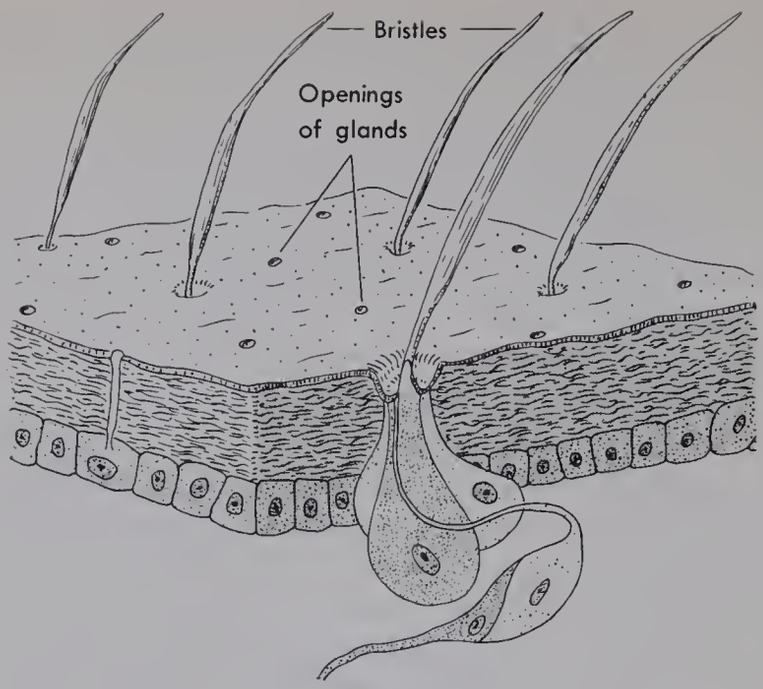


Fig. 22.8 Both the mechanoreceptors and the glands of the *Rhodnius* exoskeleton develop at evenly spaced intervals from one another.

Because of their demonstrated role in tissue repair, they will be discussed more fully in the next chapter. Whether these same substances serve as inhibitors in embryonic development remains to be seen. In any case, such a phenomenon would account nicely for the fact that as embryonic development proceeds, developing organs become sharply separated from one another. It could also account for the uniform distribution of repeating units in many organisms. For example, the body surface of the larval "kissing bug" *(Rhodnius)* is covered with evenly spaced mechanoreceptors and glands (Fig. 22.8). If a group of these in one area is destroyed (for example, by applying a hot needle to the body surface), replacement cells eventually become differentiated in the same location. These reconstruct the receptors and glands with their normal spacing. No two receptors or glands develop right next to each other. Perhaps as each begins to develop, it gives off an inhibitory substance which prevents nearby cells from developing in the same way.

Reciprocal interactions in development

It is becoming increasingly clear that the course of embryonic development depends not just on the induction or inhibition of some cells by other cells but on *interactions* occurring between both groups of cells. For example, in order for the mesoderm within the developing limbs of an embryo to become differentiated, it must have functioning ectoderm above it. On the other hand, continued differentiation of that ectoderm depends upon the continued activity of the underlying mesoderm. Perhaps, then, normal embryonic development depends not so much on the passage of a specific stimulating or inhibiting substance from one tissue to another but on the proper exchange of materials between the two.

The development of mouse kidney tubules requires two kinds of differentiated cells. One kind makes up the collecting portion of the tubule; the other, the secretory portion. It is possible to grow, in tissue culture, the embryonic cells that normally produce each of these differen-

tiated types. When grown alone, they fail to do so, even when supplied with extracts of the other cell type. But when the two are allowed to grow together, each proceeds to differentiate into its adult form. Not only do they differentiate successfully, but the two kinds of cells then proceed to organize themselves into recognizable kidney tubules.

Cells recognize each other

When, however, cells from two *unrelated* adult tissues are cultured together, the two kinds sort themselves out and unite, like with like, to form recognizable tissues. Some of these have even been grafted successfully into adult bodies and have functioned properly.

The ability of two like cells to recognize each other depends upon the presence of specific substances on their surfaces. As we have seen, embryonic cells migrate extensively, especially during morphogenesis. When they finally reach their destination, they adhere to other such cells, presumably by means of the mutual stickiness of these surface substances. Once together, portions of their respective cell membranes may fuse so tightly that an easy path is formed for the rapid passage between them of ions, electrical charges, and probably large molecules. Thus each cell has a means of knowing what is happening in adjacent cells. Interestingly enough, cancer cells lack the sticky cell-surface substances and do not recognize other cells nor stop their migration and cell division. Thus they escape from the controls that regulate the normal cells of the body.

22.8 SELECTIVE TRANSLATION OF THE GENETIC CODE

Although the inducing substances that pass from one cell to another may be quite specific or quite general in their instructions, it seems clear that what a differentiating cell does is programmed by its own genes, not those of adjacent tissues. An experiment by one of Spemann's students, Oscar Schotté, vividly demonstrated the truth of this important point.

The fate of an embryonic cell can be altered but only in a direction for which it already has a genetic program

The larval salamander has bony teeth, and a balancing organ on each side of the head. The tadpole of the frog has horny teeth, and external gills at the sides of the head. Schotté carefully grafted a piece of tissue, destined to become belly skin, from a frog embryo to the part of a salamander embryo that would normally develop into mouth and balancers. The grafted frog tissue underwent development appropriate to its *new* location, but the mouth that was produced had the horny jaws characteristic of tadpoles. Instead of balancers, external gills were formed.

An inducing substance produced by the salamander must have been responsible for changing the fate of what would have been frog belly tissue. However, the instructions provided by the inducing substance must have been quite general. Instead of programming the development of salamander parts, it seems only to have instructed the transplanted tissue to use its own genes, although in a way appropriate to the new location. Thus we must look for the ultimate control of differentiation not in any *organizer* tissue but in the reacting tissue itself. When one considers the large number of substances, even such simple molecules as methylene blue, that can induce neural plate formation in the frog embryo, it begins

to look as though the reacting tissue contains all the information required to specialize and simply needs some fairly unspecific influence to enable it to do so. The problem then becomes one of determining what makes a developing cell express certain of its genes (e.g. those participating in building horny jaws or external gills) and not others (in our example, those for belly skin).

Some workers have suggested that during the process of development, the genes contained in the original zygote nucleus become separated and are parceled out to the cells of the various tissues and organs. In Schotté's experiment, however, this is certainly not the case. The cells destined to become belly skin still had the genes for building jaws and gills. Furthermore, chromosome counts of adult cells are generally identical to those of the zygote. In Section 22.9 we shall examine additional evidence that differentiated cells retain the complete genetic library of the organism.

How, then, are the genes within a cell controlled so that certain of them are active and the rest are not? Attempts at answering this question are being made in laboratories around the world. Curiously enough, the first clues came from studies on an organism that has no embryology and no differentiation: the intestinal bacterium *Escherichia coli.*

1. The Jacob-Monod Hypothesis

Within its tiny cell, *E. coli* contains all the genetic information it needs to metabolize, grow, and reproduce. As we saw in Chapter 16, it can synthesize everything it needs from glucose and a number of inorganic ions. We would expect it to need a large number of enzymes to accomplish so many syntheses, and there may well be 600–800 present in the cell under these conditions. Most of these enzymes, such as the enzymes of cellular respiration, are present at all times. Others, however, are produced only when they are needed by the cell. For example, if the amino acid arginine is added to the culture, the bacteria soon stop producing the nine enzymes previously needed to synthesize arginine from intermediates produced in the respiration of glucose. In this case, then, the presence of the *products* of enzyme action *represses* enzyme synthesis.

Repression of enzyme synthesis in E. coli

Conversely, adding a new *substrate* to the culture medium may *induce* the formation of new enzymes capable of metabolizing that substrate. For example, if the disaccharide lactose is substituted for glucose in the culture medium, three new enzymes involved in lactose metabolism begin to appear within a few minutes. One of these enzymes, called a **permease,** transports the lactose across the cell membrane from the medium into the interior of the cell. A second enzyme, β-galactosidase, hydrolyzes the lactose molecule into the monosaccharides glucose and galactose. Once induced by the presence of lactose, the quantity of β-galactosidase in the cell rises rapidly from virtually none to approximately 3% of the weight of the cell. (The significance of the third enzyme in the metabolism of the cell is not yet known.)

Induction of enzyme synthesis

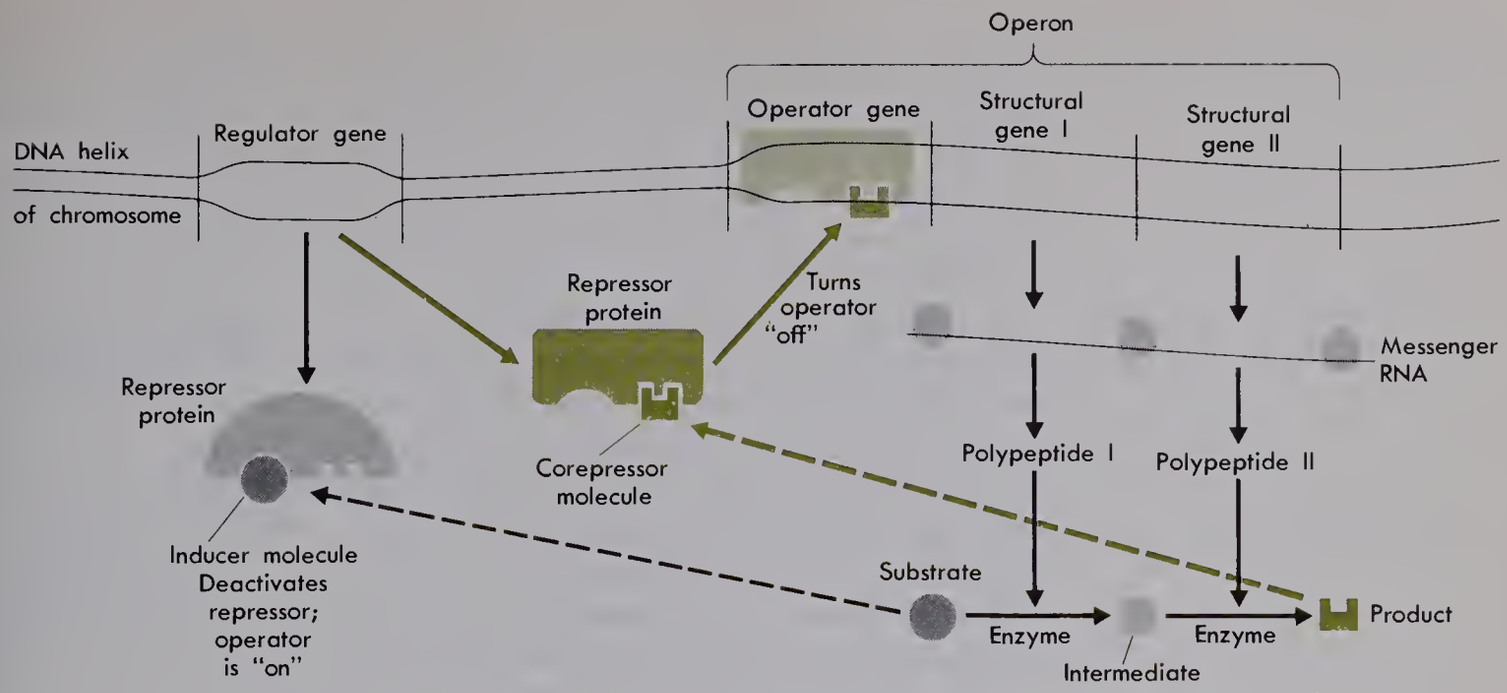


Fig. 22.9 The Jacob-Monod hypothesis of gene control. When an inducer molecule is present, the repressor is unable to attach to the operator gene and polypeptide synthesis takes place. When a corepressor molecule is present, the operon is shut down (color).

Structural genes

The synthesis of each of these enzymes is controlled by a specific gene. Genetic studies show that these so-called **structural genes,** which clearly are related in function, are also closely linked on the bacterial chromosome. What causes them to begin the work of producing their enzymes? Or rather, what *keeps* them from producing the enzymes before the need arises?

Regulator genes produce repressors

Another gene, called a **regulator gene,** is responsible for this. There is good evidence that the function of the regulator gene is to produce a protein which prevents the structural genes for β-galactosidase, etc., from expressing themselves. Curiously enough, the regulator protein, called the **repressor,** seems not to inactivate the structural genes directly; rather, it represses a gene immediately adjacent to them, called the **operator** (Fig. 22.9). The combination of the operator and its associated structural genes is called the **operon.** Perhaps the role of the operator, when it is not repressed by the regulator, is to separate the two strands of the DNA of the structural genes so that one strand can be transcribed into a single molecule of messenger RNA. Ribosomes moving down this molecule would *translate* the messages into the polypeptides from which the three enzymes are constructed. (You can see why punctuation codons—UAA, UAG, or UGA—would thus be needed to terminate polypeptide synthesis between the portions of the messenger RNA coding for each of the three enzymes.)

The operator gene

What, then, determines whether the repressor substance produced by the regulator gene is active or not? In the case described, it is probable that the presence of lactose itself prevents the repressor from acting on the

Inducers block the action of repressors

operator. Lactose may unite with the repressor, a protein, and as a result, the shape of the protein may be changed enough so that it can no longer combine with and thus inactivate the operator. The synthesis of β-galactosidase and the other two enzymes may therefore begin.

The mechanism described here was proposed by the French scientists François Jacob and Jacques Monod to explain the genetics of enzyme induction. For this work they shared in a Nobel Prize in 1965.

As we saw above, the action of some genes is repressible. The nine genes involved in the production of the enzymes for arginine synthesis turn out to be clustered in six separate operons. However, a single regulator gene responds to the presence of arginine and represses all six operons. Presumably the regulator gene produces a repressor which blocks all six operators when the repressor has combined with arginine, its **corepressor.** The usefulness to the cell of such a mechanism is quite clear. The presence of an essential metabolite turns off the synthesis of the enzymes for its own manufacture and thus stops unnecessary protein synthesis in the cell.

Corepressors

As its name suggests, the repressor is essentially a negative control mechanism. Recently, it has become evident that gene transcription in bacteria may also be under positive control. We have seen that an invading bacteriophage takes over much of the metabolic machinery of the host cell and uses this machinery to make many copies of itself. When *E. coli* begins transcribing and translating the genes of one of its bacteriophages (called T4), one of the first phage proteins synthesized combines with the bacterial RNA polymerase. In so doing, it reduces the efficiency with which the RNA polymerase can transcribe host genes and greatly *increases* the efficiency with which it transcribes other *bacteriophage* genes.

Positive gene control mechanisms also exist

Many bacteria (but not *E. coli*) form resting spores when environmental conditions begin to deteriorate. In a real sense, this represents a developmental step and thus the expression of genes not hitherto active. Interestingly enough, the molecular structure of the bacterium's RNA polymerase changes at this time, with the result that it switches its transcription from the genes associated with vegetative growth to those responsible for spore formation.

Here, then, are two mechanisms by which the genetic code in bacteria can be *selectively* transcribed and translated. Do similar mechanisms exist in higher organisms, that is, organisms that pass through stages of embryonic development and cellular differentiation? Undoubtedly they do, but despite heroic research efforts, proof of the existence of operator and regulator genes in higher organisms has not yet been secured. Different RNA polymerases have been found in different places and at different times in higher organisms, but whether they are restricted to the transcription of certain genes or sets of genes is not yet clear.

Do eukaryotes have regulator and operator genes?

2. Changing Patterns of Gene Action

In a bacterium, the genes are turned off and on in quick response to the changing needs of the cell. In embryonic development, the repression and

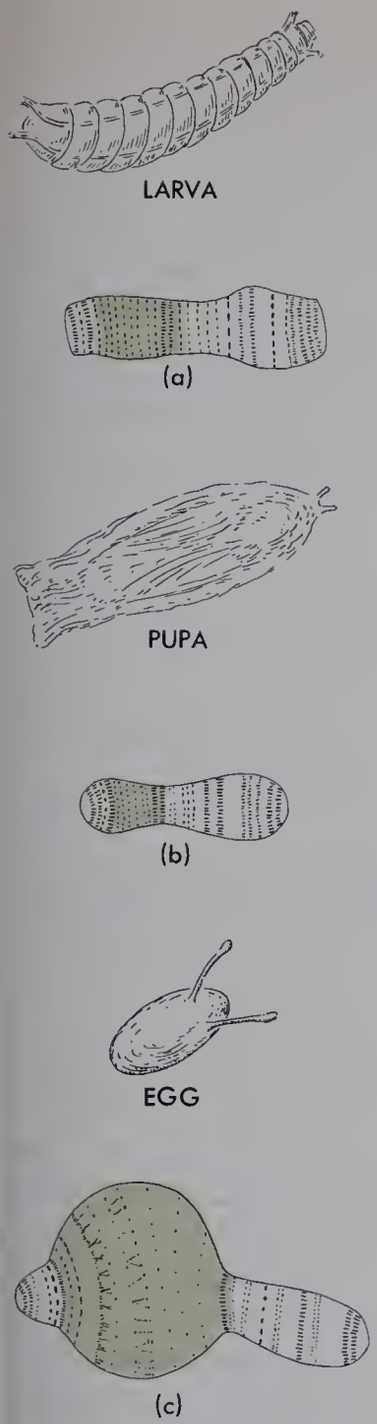


Fig. 22.10 Changes in equivalent portions of a giant chromosome of a fruit fly (a) in the larva, (b) in the pupa, and (c) when transplanted into an egg. (Eggs do not normally have giant chromosomes.) Enlargements ("puffs") in the chromosomes are associated with increased activity.

derepression of genes appears to be a more complex process. Morphogenesis and differentiation require an orderly, sequential program of gene repression and derepression. The genes concerned with the synthesis of certain proteins in the fully differentiated cell cannot do their work unless that cell has passed through a number of earlier stages of selective gene activity.

Although the Jacob-Monod theory may not be directly applicable to the problems of embryonic development, some mechanism for selectively controlling the action of the genes is surely at work. Microscopic examination of the giant chromosomes found in the salivary glands of the fruit fly (Fig. 18.3) has provided some evidence of this. It has been shown that these chromosomes actually change their structure during the course of embryonic development. They develop enlarged regions, called "puffs," and it is these regions which are most active in RNA synthesis (Fig. 22.10). Inasmuch as messenger RNA synthesis is the first step in gene action, we may conclude that the enlarged regions of the chromosomes indicate genes or sets of genes that are especially active.

The exact location of the puffs varies over the course of the differentiation of a single kind of cell and also varies from one kind of cell to another. Transplanting a nucleus to another kind of cell or to the same kind of cell at a different stage of development leads to the disappearance of its characteristic puffs and the appearance of new ones. This, then, provides visible evidence of a sequential pattern of gene activity during the course of the differentiation of different cell types (Fig. 22.10).

The evidence would be more direct if the presence of a particular puff could be associated with the presence of a single, specific gene product. Fruit flies are not the only insects that have giant chromosomes. Midges (genus *Chironomus*) have them in the cells of their salivary glands as well as in other tissues. These produce puffs which, in a number of cases, are specific to a given type of cell. Among the species of *Chironomous* are two whose salivary-gland cells differ in a clear-cut way. One (*C. pallidivittatus*) produces certain granules in some of the cells of its salivary glands, whereas the other (*C. tentans*) does not. Furthermore, *C. pallidivittatus* has one extra puff in the chromosomes of these cells that *C. tentans* lacks. Cross-breeding has shown that both these features are inherited as single gene traits and, in fact, chromosome mapping (see Section 18.6) shows that the gene is located at the point on the chromosome where the puff that distinguishes one species from the other is located. Genes code for proteins, and we would expect *C. pallidivittatus* salivary-gland cells to contain a protein not found in *C. tentans*. Fractionation of the proteins produced by these cells reveals six shared by both species and one found in *C. pallidivittatus* only. Here, then, is powerful evidence that a single gene locus produces an RNA-synthesizing puff from which are derived a unique protein product and a unique, visible feature (the granules) in the cell.

RNA synthesis also takes place in the less darkly staining regions of the giant chromosomes, that is, in the regions between the dark bands.

Here the DNA seems to be in a tenuous, extended state. The dark bands themselves contain a much greater quantity of DNA, but they do not seem to be active in RNA synthesis. Perhaps the DNA in these regions is too tightly coiled and folded to serve as a template for the synthesis of messenger RNA. Perhaps the proteins with which it is united prevent it from being active. You remember that the chromosomes of higher organisms consist of both DNA and protein. Prominent among the protein molecules are several called **histones.** There is ample evidence that the presence of histones on a chromosome blocks the transcription of DNA into RNA. When they are removed from the chromosome, the rate of RNA synthesis increases markedly. Could they selectively lock and unlock portions of the genetic code during embryonic development? Could they serve as specific repressors of gene activity?

Histones repress gene activity

This is an attractive hypothesis but unfortunately the histones do not appear to be diverse enough to carry out such a function. There are only a few kinds of histones in a cell and these are very similar from tissue to tissue and even from species to species. In fact, one type of histone found in calf cells differs from a similar histone found in *pea* cells at only two positions (amino acids) out of a total of 102 in the molecule!

Faced with the molecular uniformity of histones, some workers have suggested that histones acquire the ability to recognize the base sequence of specific genes (and then inactivate them) through some form of chemical modification, including complexing with RNA molecules. Certainly, an RNA-protein complex could easily account for the precision of gene repression because the RNA portion could carry just the codons needed to "recognize" a given sequence of bases in DNA. Indeed, there is evidence of a fourth kind of RNA, chromosomal RNA, that is found attached to chromosomes. Whether it does the job suggested, though, is an open question. Actually, there is no compelling need for it to do so. It is quite clear that proteins alone can recognize specific DNA sequences (for example, the repressor of the lactose operon). There are proteins other than histones associated with the DNA of chromosomes, and perhaps the search for negative and/or positive control mechanisms will finally lead to them.

RNA can recognize complementary sequences in DNA

Proteins can also recognize particular sequences in DNA

How, then, do molecules that induce or inhibit embryonic development do their work? Unfortunately, we simply do not yet know the molecular mechanisms involved. Macromolecular inducers or repressors could, in principle, serve directly as postive or negative control elements over gene transcription or even gene translation. But as yet there is no clear evidence that they do so. As for small molecules with inductive properties (e.g. certain steroids, methylene blue), they *could* interact with protein repressors in a fashion similar to the interaction of lactose with the lactose operon repressor. On the other hand, there are any number of other mechanisms by which arrival of a given chemical at a cell could ultimately lead to a change in the expression of the genetic material of that cell.

Clearly, attempts to find out what controls the development of living organisms have raised more questions than they have answered. Only time will tell whether the Jacob-Monod hypothesis will be able to account for

the selective and sequential transcription of the genetic code that occurs during embryonic development. Whatever the outcome, it will have played a crucial role in suggesting new experiments and posing new questions.

22.9 THE REVERSIBILITY OF DIFFERENTIATION

Embryonic development entails a decreasing number of options

As embryonic development proceeds, the ability of bits of embryonic tissue to have their future altered by transplantation to new locations becomes more limited. Their cells become progressively less flexible in their potentialities. Once gastrulation begins, for example, the cells of one tissue layer generally cannot be converted into those of another. The conversion of potential belly skin into mouth parts or the lens of an eye (Fig. 22.7) simply involves a shift from one kind of ectoderm to another. Later, the number of alternatives open to differentiating cells decreases still more. Finally, the cells become committed to a definite fate. If transplanted after this time, they continue to develop as they would have in the old location, not in a way appropriate to the new one. For example, if the limb buds are removed from one salamander embryo and grafted onto a second, they form extra legs on the host. This occurs even though the cells of the limb bud are not visibly differentiated.

The fully-differentiated cell expresses only a small fraction of its DNA

This progressive loss of developmental flexibility seems to run parallel to a progressive reduction in the amount of DNA in the cell that is active. In embryonic pea cells, about 80% of the DNA is complexed with histone and thus inactive. The remaining 20% is active in RNA synthesis. In the fully differentiated cells of the pea, on the other hand, the amount of active DNA drops to 10% or less. In differentiated animal cells, the value is probably closer to 1%. In fact, one of the most fully differentiated, and thus specialized, cells of the chick is the red blood cell. It really has only one protein to synthesize, hemoglobin. Virtually 100% of the DNA in the nucleus of chick RBC's is complexed with histone and carries on no RNA synthesis. As for mammals, you remember (see Section 13.12) that they do not even retain the nucleus once their RBC's are fully differentiated.

Differentiated plant cells still retain the entire genetic library of the organism

Are these progressive losses in the potentialities of differentiating cells utterly irreversible? Probably not. For plants, in fact, one can answer with greater certainty simply by examining Fig. 22.11. A fully differentiated carrot root cell when grown in a suitable culture medium divides repeatedly, losing its differentiated structure as it does so. Then its descendants begin, themselves, to differentiate and they finally form all the organs of a mature carrot plant. Probably any differentiated plant cell, if placed in surroundings resembling those found in endosperm (Fig. 22.3) can revert to an embryonic type and have all the genetic capabilities of its species once more unlocked.

The situation is not so clear with respect to animals. Attempts have been made to answer the question by means of nuclear transplant experiments. Using micromanipulators equipped to operate on single cells, Robert Briggs and Thomas King succeeded in removing nuclei from the cells



Fig. 22.11 The carrot plant in the flask grew from fully differentiated carrot root cells that had been isolated and induced to undergo mitosis. (Courtesy of Roy De Carava and *Scientific American.*)

The experiments of Briggs and King

of frog embryos in various stages of development. To test the potentialities of these nuclei, they transplanted them into unfertilized frog eggs whose own nucleus had been removed. In this way, it could be determined whether a nucleus from the later stages of embryonic development was fully equivalent to the nucleus of the zygote or early cleavage cells. It was soon found that nuclei from frog blastulas still retain all the potentialities of the zygote nucleus. The nucleus from any one of the thousands of cells of the frog blastula initiates perfectly normal development when transplanted into a frog egg lacking its own nucleus.

This is understandable because there is good evidence now that the chromosomes of cells during cleavage have not begun to carry out any function except their own duplication during the interphases of the repeated mitotic divisions. All the control of the activities of the cleaving cells appears to be provided by the many messenger RNA molecules and ribosomes that were deposited in the unfertilized egg by the mother during oogenesis. In other words, the genetic code supplied by the father in the sperm cell seems to play no role during cleavage. It is only at the start of gastrulation that nucleoli form, new ribosomes are manufactured, and vigorous RNA synthesis—using the maternal *and* paternal codes—begins.

Another example of cytoplasmic control of nuclear activity

The absence of substantial RNA *synthesis* prior to gastrulation probably represents another example of the influence of the cytoplasm on the genetic activity of the nucleus. When nuclei from the late stages of embryonic development, or even from young tadpoles, are transplanted into eggs,



Fig. 22.12 South African clawed frog that developed from an egg whose own nucleus had been replaced with a nucleus taken out of a fully differentiated epithelial cell from the intestine of a tadpole. This animal was fertile and normal in every other way, thus demonstrating that no part of the genetic library of the species was lost during differentiation. (Courtesy of J. B. Gurdon and *Scientific American.*)

they quickly (within 40 minutes) lose their nucleoli and stop synthesizing RNA. Not until they have guided the embryo as far as gastrulation do they resume RNA synthesis. Attempts to isolate the cytoplasmic inhibitor of RNA synthesis indicate that it is a relatively small molecule.

When Briggs and King repeated their experiments with nuclei from later embryonic stages, quite different results were obtained. These nuclei were also capable of initiating embryonic development, but with widely varying degrees of eventual success. Many of the resulting embryos ceased developing at one stage or another. Furthermore, those that did develop were often abnormal. This evidence *suggests* that during gastrulation cell nuclei do become altered. Perhaps some of their genes have become permanently repressed. However, the lack of success with these nuclei may simply reflect a failure of experimental method rather than an altered property of the nuclei. Working with the South African clawed frog, *Xenopus laevis,* Gurdon was able—in a modest percentage of cases—to grow fertile adult animals from eggs whose own nucleus had been replaced by a nucleus taken from a fully differentiated intestinal cell of a tadpole (Fig. 22.12). Thus perhaps the nuclei from fully differentiated nondividing cells are simply more easily damaged during the manipulations, and are not irrevocably repressed.

Gurdon showed that the nucleus of a fully-differentiated animal cell still contains and can be made to express the entire genetic library of the organism

The chromosomes of the chick red blood cell appear to be permanently repressed: little or no RNA synthesis can be detected. However, when chick red cell nuclei are introduced into the cytoplasm of an actively metabolizing mammalian cell by the technique of somatic cell hybridization (see Section 18.8), the chromosomes are derepressed and become active once again in RNA synthesis.

EXERCISES AND PROBLEMS

1. From what embryonic germ layer is the optic nerve derived?
2. Distinguish between identical and fraternal twins.
3. How do we know that the liver of a frog is derived from endodermal cells?
4. A tadpole ready to hatch is slightly larger than the fertilized egg was. What might account for this?
5. Female armadillos always give birth to four offspring of the same sex. Can you account for this?
6. Would you expect mutations in *regulator* genes to be inherited as dominants or recessives? Explain.
7. Would you expect mutations in *operator* genes to be inherited as dominants or recessives? Explain.

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23 DEVELOPMENT: GROWTH, REPAIR, AGING, AND DEATH

Growth

Repair

Aging—the Facts

Aging—the Theories

Death

23.1 GROWTH

Growth in animals generally follows completion of embryonic development

Plant and animal development is not limited to morphogenesis and differentiation. Development also includes an increase in the size of the organism, that is, growth. Among animals, most growth takes place after the completion of morphogenesis and differentiation. These processes are essentially completed in the human embryo by the time it reaches a weight of 1.5 grams. This weight may, however, be increased some 45,000 times before the adult size is reached. You remember also that the newly hatched frog tadpole is little larger than the original frog egg.

In plants, there exists considerable overlap in these vital developmental activities. Apical growth (in the region of elongation of roots and stems) precedes morphogenesis and differentiation. However, growth of stem thickness occurs by cell enlargement after morphogenesis and differentiation have taken place.

During growth, the rate of anabolism exceeds that of catabolism

For growth to occur, the rate of synthesis of the complex molecules of the organism, proteins for example, must exceed the rate of their breakdown. This means that additional supplies of organic molecules (e.g. amino acids, fatty acids, glycerol, and glucose) must be taken in by the organism from its environment. Some of these materials will provide the building blocks for the anabolic reactions. The others will supply the extra energy needed to run them. In photosynthetic organisms, light supplies the energy for anabolism and inorganic molecules are the raw materials.

Growth thus involves gaining more material from the environment than is given back to it in the form of metabolic wastes. This is not just a simple process of accumulation. The amino acids which you assimilate after a meal of beefsteak are not reassembled in your cells into *beef* protein. Instead, you grow by converting relatively unspecific organic molecules taken in from your environment into the specific cell materials that are characteristic of *you*.

The autotrophic organisms are even more remarkable in this respect. In growing, they convert inorganic substances from their environment (e.g. H_2O, CO_2, and nitrates) into the specific structures that form more maple tree, corn plant, etc. This ability of all living things to build more of their own specific, complex organization from relatively simple, disorganized materials in their environment is the ability to grow. It is one of the most characteristic features of life.

Growth in organisms may occur simply by an increase in the number of cells that make up the organism. The adult human is made up of some 60 trillion (6×10^{13}) cells, while the newborn baby contains only about two trillion (2×10^{12}). Growth may also occur as a result of an increase in the size of the cells that make up the organism. Monocots, lacking a cambium, grow in thickness this way. In general, though, organisms grow by both an increase in the number of cells *and* an increase in their size. Growth in woody dicots involves both activities. Tiny new cells are produced by the cambium and apical meristems. These cells then enlarge and differentiate.

The phases of growth

Several periods can usually be distinguished in the growth of an organism (Fig. 23.1). The first period, the **lag period,** is characterized by little or no actual growth. During this period, however, the organism is getting prepared for growth. A seed imbibing water, preparatory to germination, and an *E. coli* cell synthesizing the enzymes with which to metabolize its substrate are both in the lag period of growth.

The lag period is followed by the logarithmic or **exponential period** of growth. In this period growth begins, slowly at first, but then more and more rapidly. This gradually accelerating rate of growth is understandable when you remember that in most cases, the product of growth, living material, is itself capable of further growth. Thus the organism enlarges according to a geometric progression, doubling and redoubling in size. Such progressions are expressed in algebra by exponents (logarithms); hence this phase of growth is called the exponential or logarithmic phase. Different organisms vary greatly in the time that it takes them to double their size. The newly hatched housefly larva doubles its weight in 13 hours. The newborn baby usually requires 5 to 6 months to do the same. Whatever the amount of time required, all organisms pass through a constantly accelerating period of growth.

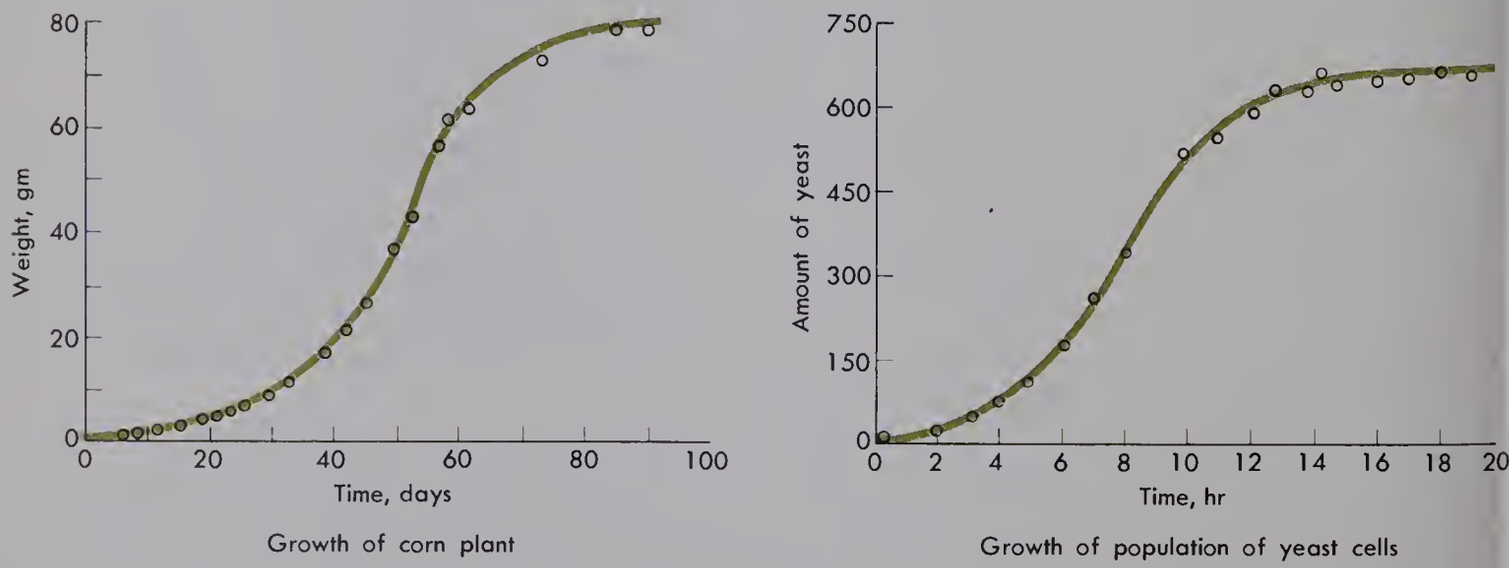


Fig. 23.1 The *S*-shaped curve of growth occurs in both individuals (left) and populations (right).

Many fishes and reptiles continue to grow throughout their lives

The exponential phase of growth does not continue indefinitely, and it is a good thing that it does not. The housefly larva, doubling its weight every 13 hours, would be an awesome creature indeed after a few days. Fortunately, the fly larva (as well as all other growing organisms) soon enters a period of **decelerating growth.** Growth now proceeds more slowly and finally ceases altogether. At this time the larva prepares for metamorphosis. In many organisms the rate of growth slows down but never ceases entirely. Many fishes and reptiles continue to grow year after year, al-

though more and more slowly, until finally they die. A similar situation occurs in trees, which continue to grow until disease or accident strikes them down.

Mammals, including man, reach a certain size and then cease growing entirely. At this point, the rate at which more living substance is synthesized by anabolism is exactly counterbalanced by the rate at which it is broken down catabolically. In later years, humans may even enter a period of negative growth, that is, they shrink. Body constituents are then broken down more rapidly than they are synthesized.

The S-shaped curve of growth

If one plots a graph showing the growth of a single organism during the accelerating and decelerating phases, an S-shaped curve results (Fig. 23.1). It is interesting to note that this same curve expresses the growth of populations. If a few bacteria or a pair of rabbits are placed in a suitable and uncrowded environment, they will produce offspring. These offspring will, in turn, produce their own offspring. This will go on in an ever-expanding way until external conditions force a slowdown in the rate of growth of the population. Biologists have a good idea of what forces are involved in the stabilizing of population growth. We shall consider these in Chapter 32. Biologists are not at all sure which forces cause a slowdown in the growth of individuals. Perhaps the controlling factors differ from one group of organisms to another. In any case, until these factors are identified, a large gap will remain in our knowledge of development.

Growth in plants

Growth in plants occurs chiefly at the meristems. Here rapid mitosis provides the necessary additional cells. In Section 14.4, we examined the way in which the apical meristem in the embryonic region of the root provides new cells which then elongate and differentiate. A similar pattern of vertical growth occurs at the tips of stems. Mitosis in the apical meristem (Fig. 23.2) of the shoot apex or terminal bud produces a stockpile of new cells. Periodically these give rise to leaves. The point on the stem where the leaves develop is called a **node** (Fig. 23.3). The distance between the nodes (the internode) in the terminal bud is very short, and the leaves develop rapidly. As a result, the leaves grow above the apical meristem that produced them and thus protect it.

New meristems, the lateral buds, develop at the nodes, each just above the point where a leaf is attached. When the lateral buds develop, they produce new stem tissue and thus branches are formed.

Under special circumstances (which we shall study in the next chapter), the apical meristem is converted into a flower bud. This develops into a flower and thus enables the plant to carry on sexual reproduction. The formation of a flower bud "uses up" the apical meristem so that no further growth of the stem can occur at that point. Branching may, however, occur from lateral buds directly behind the flower (Fig. 23.3).

In dicots, growth in diameter is accomplished by a band of cambium which separates the phloem from the xylem in both the root and the stem (Figs. 14.2 and 14.3). Except for a few palms and lilies, monocots lack a cambium. As we noted earlier, increase in their stem diameter can occur only by cell enlargement.

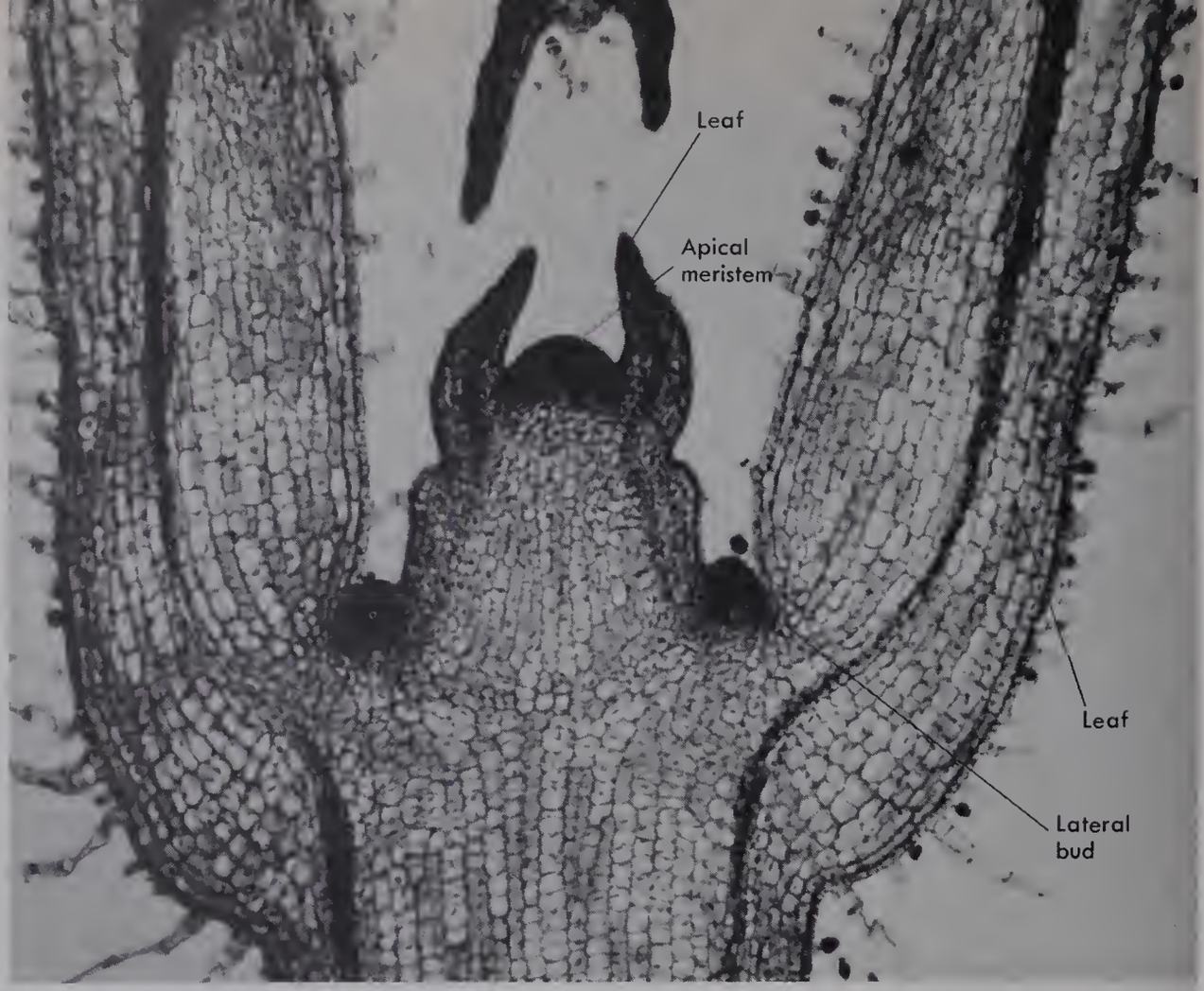


Fig. 23.2 Terminal bud of a coleus stem, cut lengthwise. New cells are produced by mitosis in the apical meristem. Later, branching may occur at the lateral buds.

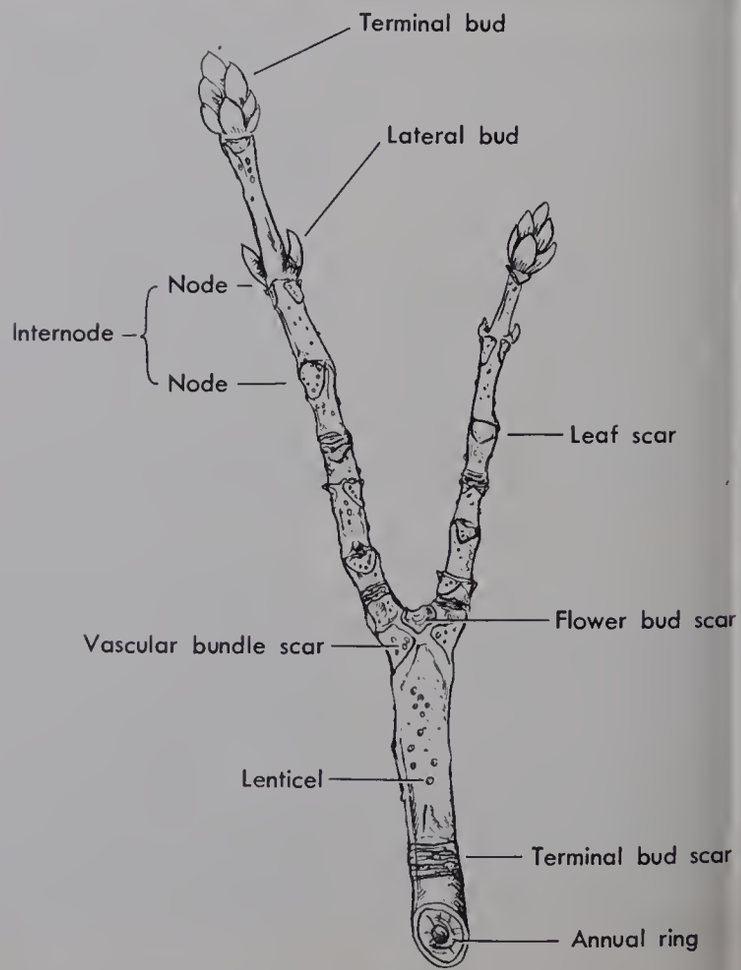


Fig. 23.3 External structures of a typical woody dicot stem, the horse chestnut, as seen during the dormant season.

Growth in animals

Growth in animals does not occur in such localized areas as it does in plants. All of the tissues and organs of the animal body participate in growth. They may not all grow at the same rate, however. Figure 23.4 shows how much faster the trunk and limbs of the human grow from infancy to adulthood than the head. The result is a marked change in body proportions.

Fundamental to the growth of the body is growth of its supporting skeleton. Bones are able to grow and lengthen only so long as they have a cartilaginous, nonbony region where further cell division and elongation can occur. In the long bones, this occurs at the epiphyseal lines just back of the hard ends of the bones (Fig. 5.18).

In humans, the growing regions of the bones become fully "ossified" during the late teens and early twenties. The cartilaginous matrix of these regions becomes replaced with a bony matrix and further skeletal growth ceases. Cessation of growth also occurs in the other mammals but usually at a younger age. On the other hand many of the fishes and reptiles continue to exhibit skeletal growth throughout their lives.

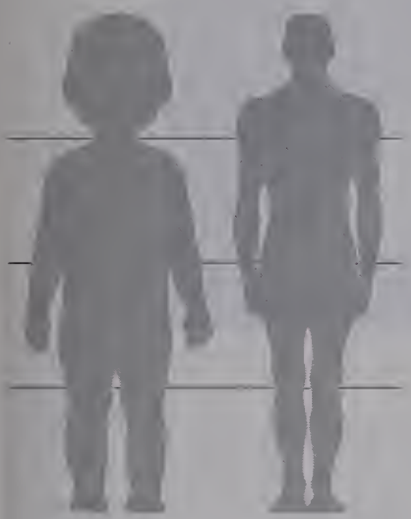


Fig. 23.4 Differential growth in the human. The head of a baby is roughly one-fourth the length of the body, while in the adult the fraction is closer to one-eighth.

The problem of development is particularly interesting in those animals that undergo metamorphosis. Most aquatic invertebrates (e.g. starfish and barnacles), many aquatic vertebrates (e.g. frogs), and most insects develop from the zygote into a **larva.** Later the larva undergoes the drastic changes of metamorphosis and the adult body is produced.

The significance of this two-stage method of development is not entirely clear. For those animals, such as barnacles, whose adult bodies are sessile (anchored), free-swimming larvae permit dispersal. In cases such as the lobster and the frog, the larva serves an intermediate stage in development during which food (usually of microscopic dimensions, e.g. algae) can be taken in from the environment. This permits more growth than is provided for by the food reserves in the egg itself and enables the animal eventually to reach the size necessary for it to have the characteristic diet of the adult.

Larvae are not embryonic organisms

While larvae are a stage in the development of many animals, it is wrong to think of them as incompletely developed or embryonic organisms. For example, the garden caterpillar is a highly specialized creature. It is the product of an elaborate morphogenesis and complete cell differentiation, complete, that is, except for a few masses of embryonic cells, the *imaginal disks,* within its body. At the time of metamorphosis, most of the specialized cells of the caterpillar die. These dying cells supply the nourishment necessary for the cells of the imaginal disks to undergo an entirely new pattern of morphogenesis and differentiation. The result is the adult organism, a moth or butterfly. All growth in insects occurs during the larval stages. In fact, some adult insects do not even feed. They simply mate and die.

Another reason why larvae should not be considered incompletely developed forms is that cases are known in which certain species have abandoned metamorphosis entirely. The axolotl, an amphibian found in Mexico and the American Southwest, spends its entire life as a gill-breath-

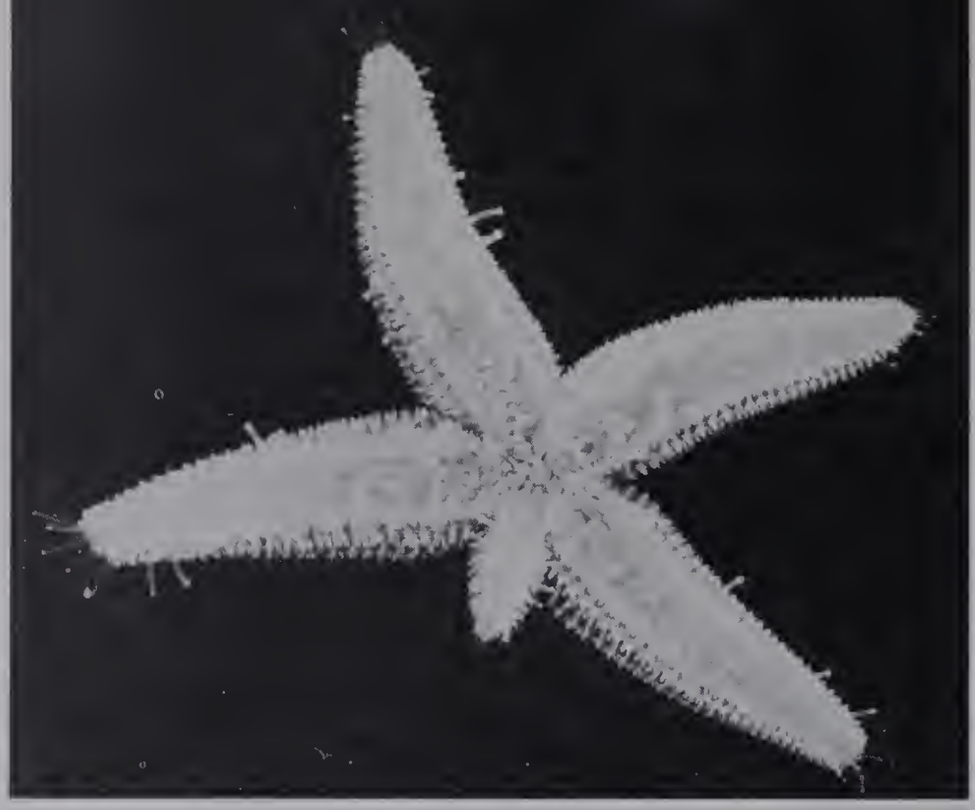


Fig. 23.5 Starfish regenerating arm. (Courtesy of Dr. Charles Walcott.)

ing animal (Fig. 25.5). It reaches sexual maturity and mates without ever undergoing metamorphosis to an air-breathing form. While there is no evidence that the axolotl is going to lead to the evolution of a whole new group of animals, there is evidence that in the past, larval, rather than adult, forms have sometimes evolved into new species. We shall examine some of this evidence in later chapters.

23.2 REPAIR

During the lifetime of an organism, some of its parts may become damaged or lost. Most organisms have, to some degree, the ability to replace defective or missing parts. This process of replacement is called **regeneration** (Fig. 23.5).

Regeneration in plants

Plants generally have great powers of regeneration. They can have many, sometimes all, of their branches and foliage removed by pruning. If the root system is healthy, however, buds soon appear on the stem or trunk. The buds develop into new branches, leaves, and flowers. In fact, many hardwoods (maples, birches, etc.) can be cut off right at ground level and sprouting at the margins of the stump will soon produce new stems and leaves.

Regeneration in animals

The ability of animals to regenerate missing parts varies greatly from species to species. Sponges can regenerate the entire organism from just a conglomeration of their cells. This is also true of the hydra. A planarian can regenerate the entire organism from a middle section. Even the starfish can regenerate an entire organism from just one arm and the central disk. At one time oyster fishermen used to dredge up starfish from their oyster beds, chop them up in the hopes of killing them, and then dump the parts back overboard. They soon discovered to their sorrow the remarkable powers of regeneration in this group of animals.

Earthworms, crustaceans, fish, salamanders, and lizards do not possess such powers of regeneration that they can regenerate the whole organism from just one part. They can, however, regenerate fairly substantial parts. The earthworm can regenerate the first four or five segments of its head and even longer sections of its "tail." Lobsters and salamanders can regenerate a missing leg. Many lizards will part with their tail if caught by it. They then regenerate a new one at their leisure. Birds and mammals cannot regenerate entire organs. They can, however, regenerate tissues and thus repair damaged or missing parts. The healing of skin wounds and bone breaks is an example of regeneration in man. The replacement of blood after blood loss is another example. The digestive glands, especially the liver and pancreas, are capable of extensive regeneration after damage.

Dedifferentiation precedes regeneration

What happens in the process of regeneration? If a foreleg is removed from a salamander, the first repair process is the healing of the wound by means of skin growing over it. Then a bud of undifferentiated cells appears. This bud has the same appearance as a limb bud in the developing embryo. The rapidly dividing, unspecialized "embryonic" cells of the limb bud probably arise from the **dedifferentiation** of such specialized cells as muscle and cartilage cells. Dedifferentiation means that these cells lose their differentiated structure prior to taking on the task of regeneration. As time goes on, the cells of the regenerating limb become organized and differentiated once again into the muscle, bone, and other tissues which make up the functional leg.

Chalones

Studies of regeneration have revealed that mature, differentiated cells of a given tissue, e.g. epidermis, synthesize and secrete a substance which actively inhibits mitosis of the young cells of the same tissue. This substance is called a **chalone.** In the early stages of regeneration, there are no mature cells and thus there is no inhibition of cell division. As the tissues of the regenerated structure become differentiated, chalone production begins and, presumably, this gradually stops the growth of the regenerated structure.

Actually, the production of chalones by differentiated cells seems to be a general phenomenon. Virtually every tissue that has been examined (including liver, kidney, lung, uterus, hair follicle, and white blood cells) produces a chalone which inhibits mitosis in the immature cells of that tissue but of *no other* tissue. The chalones do not harm the other activities of these cells in any way. Although chalones have not as yet been well characterized chemically, they behave like proteins or polypeptides. Although their action is exceedingly specific with respect to tissue, it is not so with respect to species. An epidermal chalone from one mammal (even from a fish) will inhibit mitosis in the epidermis of another mammal.

Parallels between regeneration and embryonic development

In many ways, the process of regeneration is similar to the process of embryonic development. From rapidly dividing, unspecialized cells arises a complex organization of specialized cells. This involves morphogenesis and differentiation just as embryonic development does. There is, however, at least one way in which the process of regeneration differs from the process of embryonic development. Can you think of what it is?

Polarity in regeneration

The similarities between regeneration and embryonic development have caused some embryologists to study regeneration in the hope of gaining an understanding of how embryonic development "works." The discovery of **polarity** has shown that definite organizing forces, probably chemical, are at work in regeneration. A midsection of a planarian will regenerate a new head at the same cut edge at which a head was originally present. A tail will be regenerated at the other edge. It has been found that the cells at the "forward" cut surface have a higher metabolic rate than those at the "rear" edge. It seems to be this difference in metabolic rates in the cells of the regenerating piece that determines polarity. If the rear section of a worm is removed and the head is then treated with a solution containing a metabolic inhibitor, the polarity of the worm can be reversed and a second head will form at the posterior edge (Fig. 23.6).

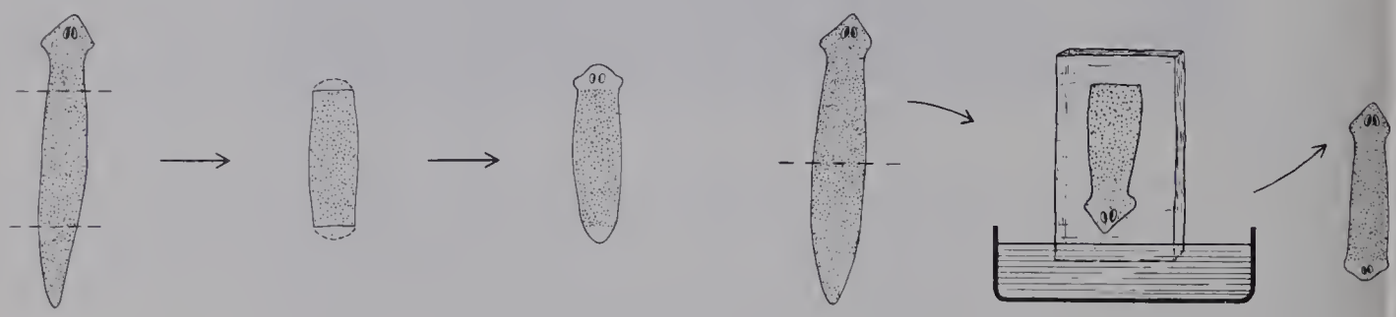


Fig. 23.6 Regeneration in planarians. Left: Each cut surface of the planarian "remembers" its polarity. Right: When the head of a cut planarian is placed in agar and dipped in water containing a metabolic inhibitor, a second head develops at the cut surface.

In comparing the regenerative abilities of different animals, there seems to be some relationship between the complexity of the organism and its ability to regenerate. The powers of regeneration in the sponge are practically complete. In man regeneration is limited to repair of certain organs and tissues. We wish we knew just why it is that regenerative powers diminish with increasing structural and physiological complexity.

The powers of regeneration usually diminish with age

Within a single organism, at least among the vertebrates, there also seems to be a progressive loss of regenerative ability with increasing age. When legs first appear on a frog tadpole, they can be regenerated easily if lost. After metamorphosis, however, a frog is normally unable to regenerate a missing leg. Everyone knows how much more quickly broken bones and skin wounds heal in a child than in an elderly person. What causes the loss of regenerative power as age increases? In the frog, it may be that the area of amputation fails to receive sufficient amounts of some substance released by the nerves. When extra nerves are moved surgically to the stump of a frog foreleg, regeneration does occur. It is known that the ratio of nerve tissue to other tissues decreases as the tadpole grows into a frog. It is also possible, though, to bring about leg regeneration in the frog simply by irritating the stump. This suggests an alternative explanation.

Perhaps aging tissue simply loses the ability to respond to the regeneration-promoting substances released by nerves. If this is the case, irritation of the tissue may restore some of its sensitivity.

Cancer. The gradual development of pattern in both regeneration and embryonic development tells us that tissues normally grow to a certain size and then stop. Further cell division in the tissue awaits, and is regulated by, the need for cell replacement (as in the replacing of old red blood cells and sloughed-off epidermal cells, or the regeneration of damaged parts). Sometimes the process fails. A cell escapes from the normal control mechanism (e.g. chalones) of the body and begins to divide repeatedly and uncontrollably. As it and its progeny continue to divide, they often lose to some degree the characteristic structure and functioning of normally differentiated cells of that type. To some extent, they begin to resemble embryonic cells.

A cancer is a clone (?) of cells dividing uncontrollably

Every cell type known can become cancerous. What converts a normal cell into a cancerous cell is not yet known with certainty. Radiation, many chemicals, and a number of viruses can bring about this cancerous change, but the exact mechanism by which they do so remains to be discovered.

23.3 AGING—THE FACTS

Aging can be defined as the progressive deterioration, with the passage of time, of the structures and functions of a mature organism. This ultimately leads to the death of the organism. Either the progressive loss of function makes the organism less able to withstand infectious disease or, often, the failure of some vital organ precipitates the death of all the rest.

The study of aging has long been neglected. But now that so many infectious diseases of humans have been brought under better control by vaccines, sulfa drugs, and antibiotics, more and more people are living to an age when the process of aging becomes quite obvious. Because of this, there recently has been a great surge of interest in the problem of aging. Doctors, social workers, physiologists, biochemists, embryologists, histologists, endocrinologists, and many others are turning their attention to this problem. Not only is aging in humans under direct study, but many other organisms are being examined for clues as to the nature of aging. As a result of these studies, one fact stands out already. The effects of increased age vary widely from one living thing to another.

Among the bacteria, the process of aging does not even occur. When a single cell is fully grown, it divides into two cells, which then repeat the process. Although a wide variety of changes in the environment may kill a bacterium, no cell has ever been known to die because of aging. For this reason, some biologists have described the bacteria as potentially immortal. They have also included all other kinds of single-celled organisms in this category. While the idea that the bacteria enjoy a kind of potential immortality may have some merit, it is probably not true that all single-celled forms can be included.

Some unicellular organisms do not age

Normal mammalian cells cannot be cultured indefinitely

At one time it was thought that removing cells, or even tissues, from animals and culturing them *in vitro* conferred an "immortality" on them similar to that enjoyed by cultured bacteria. However, the situation is not as simple as that. It is true that certain mammalian cells may be propagated indefinitely, but in every case these cells are *not* normal. They have a number of abnormalities among which are an abnormal karyotype (see Section 18.4) and, generally, the potentiality of growing into a cancerous tumor if reimplanted in the species from which they have been taken. Many successful long-term cell cultures are, in fact, derived from cancers. "HeLa" cells, for example, which are now cultured in laboratories around the world, were isolated many years ago from a human cervical cancer.

Normal mammalian cells *can* be cultured *in vitro,* but generally they pass through no more than about 50 cell doublings before the cells decline in vigor and die. Interestingly enough, cells taken from young mammals are able to pass through more generations than cells taken from old individuals. This would suggest that mammalian cells have an inherent limitation on the number of cell divisions they can accomplish, whether *in vivo* or *in vitro.*

Everyone agrees that multicellular organisms are mortal. Presumably the loss of independence which occurs when cells specialize in a multicellular organism leads to eventual death. In specializing to carry out a certain function, cells abandon other vital functions to neighboring cells. If disease or other damage should strike down any one group of specialists, all the rest will fall, too. Thus heart failure results in the speedy death of all our cells no matter how vigorous and healthy they might otherwise be.

Some organisms avoid aging by continually replacing old parts

Although all multicellular organisms eventually die, it may not be accurate to say that they all age. Some seem to avoid aging and, in the process, may achieve remarkable records for length of life. Some sea anemones are known to have lived for as long as 78 years. They may achieve their longevity and avoid aging by periodically replacing all old body parts. Woody perennials fall into a similar category. Throughout their lives, they produce new vascular tissues, leaves, and flowers each year. They do not show signs of aging, although their rate of growth does decline with advancing years. Finally, disease or inability to support their ever-increasing size against wind or snow load lead to their death. This may not occur for a long period. Tree ring analysis shows that some of the bristlecone pines of eastern California are over 4,000 years old. At the moment, this seems to be a record for tree longevity. Note, however, that no *living* cells in these trees are more than a few years old.

Many fish and reptiles also seem to avoid, or at least inhibit, aging by continuing to grow throughout their lives. We do not know just what brings on death, but it may simply be the same factors that cause death in younger members of the species: disease and predation. These challenges to life, acting in a completely random way, will eventually strike down all the members of a given generation (Fig. 23.7). It is probably also true that fish and reptiles become less well adapted to their environment when they exceed a certain size. In any case, the ability to grow steadily,

even if slowly, does seem to protect them from the harmful effects of aging. Some marine turtles are estimated to live more than 150 years.

Aging usually follows the cessation of reproduction

The situation is quite different among the annual plants (such as the grasses and many "weeds") and the mammals. Most annual plants grow vigorously for a period and then form flowers followed by fruits. Fruiting is accompanied by a marked slowing down of growth. As the growth rate slows to a halt, other changes take place, too. A great deal of chlorophyll may disappear from the leaves. The rate of respiration (and thus, catabolism) rises markedly. Many other morphological changes follow. All of these changes constitute aging and end with the death of the plant. It is important to realize that these changes are not necessarily related to adverse changes in the physical environment. Aging in annual plants may occur in the midst of plentiful moisture, soil minerals, sunlight, and warm temperatures.



Fig. 23.7 Representative survival curves.

Note:

A: Typical of organisms which (1) age and (2) suffer little random mortality before then.

B: A harsh environment (starvation, disease) obscures the effect of aging.

C: Theoretical curve for organisms which (1) do not age (turtles) or (2) suffer crushing random mortality before time of aging (songbirds in wild).

Mammals, as we have seen, also grow to a certain size and then stop. Some time after the cessation of growth, aging begins. The actual time span involved varies widely from species to species. A three-year-old laboratory rat is very old. In man, although the deterioration associated with aging can be detected by the age of 30 years, fatal loss of function may not occur until much later.

Structural and functional deterioration with age

What are the symptoms of aging? Decreased muscular strength, decreased lung capacity, decreased pumping of blood from the heart, decreased urine formation in the kidney, and decreased metabolic rate are just a few of the many body changes which occur with aging. Figure 23.8 shows still other examples of the structural and functional deterioration that occurs between ages 30 and 75.

Why do the various body organs gradually lose their ability to function well? One answer is that they lose the cells of which they are composed. Many organs of the body lose weight with age (Fig 23.8), and even those that do not, do lose the specialized cells which enable them to carry out their function. These cells may then be replaced by connective tissue or fatty tissue cells, however, and the overall weight of the organ remains constant. It is interesting to note that organs which lose so much of their ability to function (and thus show so much aging) are those organs whose cells are no longer dividing actively. These include the heart, brain, kidneys, and muscles. On the other hand, the organs in which cell division is actively maintained (e.g. the bone marrow, the liver, and the pancreas) show far less loss of function with age. The situation reminds us of the perpetual youthfulness of rapidly dividing bacterial cells.

(a) Weight of brain	56%
(b) Blood supply to brain	80%
(c) Output of heart at rest	70%
(d) Number of glomeruli in kidney	56%
(e) Glomerular filtration rate	69%
(f) Speed of return to normal pH of blood after displacement	17%
(g) Number of taste buds	36%
(h) Vital capacity	56%
(i) Strength of hand grip	55%
(j) Maximum O_2 uptake during exercise	40%
(k) Number of axons in spinal nerve	63%
(l) Velocity of nerve impulse	90%
(m) Body weight in males	88%

Fig. 23.8 Loss of structure and function in aging. Figures represent percentage of a given function remaining in an average 75-year-old man compared with that found in an average 30-year-old man, the latter value taken as 100%.

23.4 AGING—THE THEORIES

What causes the degenerative changes of aging? This is one of the many unanswered questions in biology. A great many theories of aging have been proposed, but the known facts of aging are still too few either to substantiate or discredit any of them.

The mutation theory of aging

A few biologists feel that aging is largely a result of adverse changes in the environment. They are particularly concerned with the effects of radiation (e.g. cosmic rays, x-rays) on the genes in the body cells or somaplasm. Mutations in the somaplasm are not as easily detected as mutations in the germplasm. (Virtually the whole science of genetics has been based on the latter.) There is no reason to think, though, that the DNA in body cells is appreciably more resistant to mutations than the DNA in the germ cells. Remembering the one gene–one polypeptide theory (see Section 19.4), we would expect body cells with acquired mutations to produce defective polypeptides and proteins. This would naturally reduce their efficiency and might even cause their death.

In support of this theory, a number of workers have demonstrated that as human cells (growing in tissue culture) begin to age, they do



Fig. 23.9 Effect of radiation on aging. These mice are all 14 months old. As young adults, 9 mice were given sublethal doses of radiation and 9 others were left as untreated controls. The control mice (left) are still sleek and vigorous at 14 months, while 6 of the irradiated mice have died and the remaining 3 show signs of extreme aging (right). (Research photograph of Dr. Howard J. Curtis.)

produce defective enzymes. Aging vinegar "eels" (actually tiny worms) and even aging *Neurospora* cultures do the same.

It has been shown that sublethal exposure to radiation lowers the life expectancy of many animals (Fig. 23.9). However, radiation exerts its most drastic effects upon rapidly dividing cells and it is the tissues with rapidly dividing cells (e.g. liver, bone marrow) that seem the freest from the effects of aging. Cell damage and death in such tissues can be compensated for. The defective cells can be destroyed by lysosomes and phagocytes and healthy replacements produced by mitosis. In organs such as the brain and muscles, where mitosis does not occur, the damaged cells become a permanent liability.

Is aging programmed in the genes?

Many biologists feel that the tendency to age is intrinsic. They claim that even under the best environmental conditions, organisms will age at a rate determined by the nature of their genes. There is a good deal of evidence to support this view. We have seen that annual plants age despite the maintenance of suitable physical conditions around them. The widely different life expectancies from one species of mammal to another are difficult to explain without including hereditary factors. Even within a single species, some family lines show consistently greater longevities than others. The best way to assure yourself a long life is to have long-lived parents! The fact that, as a group, human females have an average life

expectancy greater than males also suggests that hereditary factors are at work. (It does not prove this, however. Why not?) Not only do human females tend to outlive the males but female rats, mice, fruit flies *(Drosophila),* spiders, and many fish do, too.

Most biologists accept the idea that aging results from an interaction of both hereditary and environmental factors. Consequently, most of the many current theories on aging assume an interaction of these two forces. But if any aspect of aging is under the influence of the genes, it means that aging is as much a part of the overall process of development as is, say, morphogenesis.

One popular theory explains aging as a consequence of living. That is, the more rapidly the organism lives, the sooner it begins to age. Rapid living is defined as rapid differentiation and growth. It also involves a high rate of metabolism. It is certainly true that mammals, such as rats, which reach maturity weeks after birth, age far more rapidly than mammals, such as man, that take years to mature.

Metabolic rate and aging

One way to test this theory is to find some way to slow down an organism's rate of development. It has been found that feeding young rats just enough to keep them alive does just this. When these rats are finally given adequate diets, they usually catch up to their normally raised cousins in almost all respects. In fact, they are apt to be living comfortably long after their well-fed cousins are dead!

The "clinker" theory

Another theory that relates aging to living has been called the "clinker" theory. According to this theory, each cell unavoidably accumulates poisonous wastes during its lifetime. The accumulation gradually reduces the ability of the cell to function, and the cell thus ages. This theory is supported by the finding of large quantities of pigment in the cells of aged people, especially nondividing cells such as those of muscles and nerves. Rapidly dividing cells, such as are found in the liver, contain much less pigment. (You remember that it is the organs in which rapid cell division does not continue throughout life that age the most.) Although the exact chemical nature of this pigment is not yet known, there is evidence that it represents the remnants of old, worn-out cell structures (e.g. mitochondria) that have been incorporated into lysosomes.

Why is the cell unable to rid itself of these harmful substances? It may be that older cells are less able to rid themselves of any substance. Collagen, the chief structural protein of the body, is found in the extracellular coatings or matrix of most of the cells of the body. When collagen is first synthesized, it is quite flexible and easily dissolved. As time goes on, the polypeptide chains of which collagen is made become increasingly linked together. This reduces the solubility and flexibility of the material. It is certainly true that organs and tissues (e.g. skin) in aging animals become less flexible with advancing age. It may also be true that aging collagen provides a barrier to the easy passage of materials in and out of capillaries and, perhaps, in and out of cells. Reduced permeability has been detected in the lungs, kidneys, and skin of aging animals. For example, when histamine is injected under the skin, the capillaries become more permeable,

The aging of collagen

and an increased quantity of protein diffuses into the tissue spaces. However, the magnitude of this response is greatly reduced in old animals. Reduced permeability would also explain why so many of the body's homeostatic mechanisms work more slowly with advancing years (Fig. 23.8).

If the aging of collagen is the clock that times aging for the entire organism, we can see why animals do not age so long as they continue to grow. Growth involves the continual synthesis of new, young collagen and thus slows the ticking of the clock.

But why should the collagen of an old rat that stopped growing two or so years before, be as old as that of a 70-year-old human who stopped growing 50 years before? Perhaps the rate of collagen aging is not just a simple function of time but also of metabolic rate. If so, this would provide a molecular basis for the theory, discussed above, that aging is a consequence of rate of living. Small mammals do, indeed, have higher metabolic rates than large ones.

You may feel dismayed that there are so many different theories to explain such a vital process as aging. Actually, this is a perfectly normal stage in the development of any science. The fewer the facts that are known, the more theories are plausible. It does not matter now which, if any, of these theories best explains the facts of aging. What is important is that each theory of aging that is concocted should suggest definite experiments by which the theory can be tested. Whether or not a given theory is successful in predicting the outcome of an experiment, it will at least have resulted in the acquisition of new knowledge. The more we know about aging, the fewer will be the theories that can explain all the facts. Only thus, by a process of closer and closer approximations, can we hope to discover the true nature of the aging process.

The role played by theories

Evolution and aging

If aging is even partly controlled by our genes, is there any hope that we can increase our life span through evolution? It is not likely. As we shall see in Chapter 32, the only traits upon which evolutionary forces can operate in a positive way are those that appear before the organism has finished raising its offspring. Unusual slowness in aging or unusual longevity will not be discovered until the chance to pass these traits on to a large number of offspring is gone. Unless the inherited traits that promote longevity also promote vigor during the years of reproduction, there is little likelihood of these traits becoming generally established in the population. In this connection, it is interesting to note how many species of living things begin aging as soon as their reproductory activities are completed.

There is at least one case where evolution has probably led to a shortening of the life span rather than a lengthening of it. Species of annual plants, whose individuals live for only one season, are probably better able to adapt to changing environmental conditions than perennial plants, because there is a complete population turnover each year. We have seen that sexual reproduction promotes variability within the species. Any one individual makes only a small contribution to the process. A 100-year-old elm tree has been producing for decades annual crops of offspring whose range

of genotypes has most likely remained unchanged. Annual plants, on the other hand, remove themselves totally from the breeding population after a new generation is formed. The next season will find the field exclusively populated by the second generation and any variations appearing will have full opportunity to meet the tests of the environment. If successful, they will play a larger role in the formation of still another generation than if their parents were still in the game, too. In view of this, it is not surprising that the annual plants are among our most hardy and adaptable plants. In fact, we call many of them weeds because of these characteristics.

23.5 DEATH

The outcome of aging is death. As the organs of the body become less efficient, the body becomes less able to cope with the stresses of life. Infections are controlled less easily. Shifts in body chemistry are corrected less easily. Finally, a given organ fails to carry out its vital functions upon which all the other organs depend. Death results.

Cessation of heartbeat

Although other organs may fail first, we mark death in man at the time the heart stops beating. When you consider the role it plays in supplying food and oxygen to the cells, this should not be surprising. Without food and oxygen the constituent cells of the body die. They do not, however, all die at once. Even after the human body is dead as a functioning organism, various cells survive for short periods of time. Nerve cells are among the first cells to succumb to lack of oxygen. Some of the skin cells are among the last. Eventually, though, every cell of the body dies.

Brain death

With advances being made in heart-lung machines and other supportive measures, it may soon be necessary to reexamine our definition of death. With such devices, it is now possible to maintain a supply of food and oxygen to all the cells of the body even though the heart or breathing has failed. What, then, can we use as a criterion of death? As we shall see in Part V, it is the central nervous system that coordinates the actions of the body. Without a functioning brain, we are little more than an assemblage of cells. Therefore, irrevocable loss of brain activity is death, of the *organism* even if not of all its constituent cells.

The death of cells is accompanied by a rapid dissolving of the cell constituents. This process of cell degradation illustrates beautifully the need for continual supplies of energy to maintain the complex organization of matter that we call life. Once energy ceases to be made available to the cells, the precise organization of their parts becomes quickly and totally destroyed.

Death as a normal stage of development

It is hard to visualize how the death of cells can also be considered a normal stage in development. Nevertheless, there are examples where cell death plays an important role in the life of the organism and presumably occurs as a result of definite genetic controls. One of the best examples is found in those insects that undergo complete metamorphosis. In the change from larva to pupa, many of the specialized cells of the larva die. These dying cells then supply the materials which enable the cells of the

imaginal disks to develop into the pupa and then the adult. This widespread death of the cells of the larva is clearly a vital stage in the overall development of these insects.

Genetically programmed death may also occur in certain fishes. The sea lamprey and the salmon are well-known examples. When they reach sexual maturity, these animals migrate from the ocean into fresh-water rivers and streams. Here the females lay their eggs, and the males fertilize them. Once the task is done, the adults die. The phenomenon is really quite similar to that of the annual plants mentioned earlier in the chapter. The removal of the old generation seems as much a part of development as the embryology that now occurs in the fertilized eggs that are the new generation.

In the past eight chapters we have examined many aspects of the process of reproduction. Central to all of these has been the nature and expression of the hereditary controls within cells. These controls, the genes, guide the development of the individual into an adult and guide their own passage to the next generation through the mechanics of reproduction. We have looked for clues as to how the genes are regulated, over time and from cell to cell, so that the organism is maintained and the species preserved. It is not enough that the selective transcription of genes be able to produce a functioning adult organism. The genes must *continue* to act selectively so that the organism can respond to changes that it will meet until death finally occurs. Each differentiated cell in the organism's body must be able to modify the expression of its genetic potentialities in response to changes occurring both outside and within the organism. In other words, the ability of organisms to cope with changes in their environment and to coordinate the multitude of activities that occur in the diverse cells of which they are composed is, in the last analysis, a matter of the regulation of gene expression. How multicellular organisms integrate the activities of their cells as they respond to changes in their environment is the topic to which we now turn.

EXERCISES AND PROBLEMS

1. Summarize the mechanisms by which the beef protein you eat is converted into proteins characteristic of you.
2. What is the average number of times every cell in the newborn baby has to divide in order to produce an adult?
3. Which best describes an actively growing organism, an arithmetic progression or a geometric progression? Why?
4. A certain restaurant buys 1000 amber water tumblers. An average of one tumbler is broken every day and each broken tumbler is replaced by a new one made of clear glass. What is the shape of the survival curve of amber tumblers?

5. Which of the following organisms would you expect to have a survival curve like that in Question 4: (a) contemporary humans in the U.S., (b) humans living in prehistoric times, (c) parrots in cages, (d) sparrows in the wild, (e) alligators in the wild?
6. What food does a tadpole eat? What food does an adult frog eat? Of what significance is this difference in diet?
7. List all the factors you can think of that affect growth in humans.
8. Which human organs regenerate damaged portions most easily? Which human organs show the fewest degenerative changes in old age?
9. How do the two lists you prepared for Question 8 compare? What explanation can you give for this?
10. In what ways are the processes of regeneration and embryonic development similar? In what way are they different?

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Belle, an adolescent chimpanzee, grooms Bandit's teeth with a small pine twig tool. Shadow (standing) observes. (Courtesy of C. E. G. Tutin and W. C. McGrew.)

Part V RESPONSIVENESS AND COORDINATION



24

RESPONSIVENESS AND COORDINATION IN PLANTS

Importance of Internal Communication

Growth Movements

The Mechanism of Phototropism

The Discovery and Role of Auxin

Other Auxin Activities

How Do Auxins Work? A Theory

The Gibberellins

The Cytokinins

Abscisic Acid

Ethylene

The Flowering Process

Factors That Initiate Flowering

Mechanism of Photoperiodism

The Discovery of Phytochrome

Other Phytochrome Activities



Fig. 24.1 The sensitive plant, *Mimosa pudica.* Darkness, touch, heat, and certain chemicals cause the leaflets to fold together along the midrib (right).

One of the chief distinguishing features of living things, as opposed to nonliving things, is that living things are capable of actively responding to certain changes in their environment. These environmental changes serve as **stimuli** which trigger a definite response on the part of the organism. When the "sensitive plant" *Mimosa pudica* is darkened, the leaflets along each side of the midrib fold together (Fig. 24.1). When the plant is illuminated once again, the leaflets spread out into their former position. The stimulus is the presence or absence of illumination; the response is the movement of the leaflets.

24.1 IMPORTANCE OF INTERNAL COMMUNICATION

Effective responses require internal communication

Responsiveness in multicellular organisms requires a proper coordination of parts. Few responses could be accomplished successfully if every cell of the organism responded in the same way to the stimulus. For you to be able to run, you must contract certain muscle fibers while others relax. Your liver must provide additional fuel for your muscles. Your lungs must supply an extra amount of oxygen and take away a correspondingly increased amount of carbon dioxide. In other words, for your entire body to respond as a whole to a given stimulus, its constituent systems, organs, tissues, and cells must respond in a variety of special ways. Furthermore, the response of each of the various parts of your body must be carefully coordinated with the response of all the other parts. This ability of individual cells, tissues, organs, and systems to respond harmoniously to the activities of other parts requires a form of communication among them.

Coordination by nerves

Two different, but related, systems of internal communication are found in animals. One of these, the **nervous system,** is a very fast-acting system. Specialized cells, **neurons,** conduct electrochemical impulses from one part of the body to another. These impulses accomplish quick, localized communication between parts. The impulses are of brief duration and quite separate from one another. Consequently, nervous responses are usually brief, intermittent, and do not continue for long periods unless the stimulus remains.

Coordination by hormones

The second communication system, the **endocrine system,** is usually somewhat slower in its action. Specialized glands, the endocrine glands, release **hormones** to the blood stream or other circulating fluid. Hormones are chemical substances which are carried by the circulatory system to every cell of the body. Sometimes just a few, sometimes many, cells respond to the presence of these hormones. Usually the response occurs as a change in the metabolic activities of the "target" cell, tissue, or organ. These changes may persist for long periods of time.

Plants differ from animals in having no nervous system. Therefore, quick, localized responses are practically nonexistent in the plant kingdom. A few plants, however, do exhibit rapid movement. When *Mimosa* leaflets are pinched, they fold up. This change occurs as a result of a sudden loss of turgor in a special mass of parenchyma cells at the base of each leaflet. If one pinches just the tip leaflets of a *Mimosa* leaf, the leaflets fold up in pairs working from the tip back to the base. It certainly looks as though some stimulus is passing up the leaf. This may be a chemical moving through the vascular bundles. Although nerves are not involved, there is also evidence that a definite electrical impulse does pass up the leaf.

Plant responsiveness is generally accomplished by hormones

For the most part, plants achieve their responsiveness and coordination through a system of chemical coordinators, the plant hormones. Our knowledge of the nature and interplay of the various chemical regulators in plants is still quite fragmentary. In this chapter we shall examine some of what has been learned from experimentation and some of the current theories on the coordinating mechanisms in plants.

24.2 GROWTH MOVEMENTS

With the exception of turgor movements, plants respond to changes in their environment by growth. Naturally this sort of response takes longer to occur than a turgor response or a nervous-system response in an animal. The growth response may consist in having one part of the plant grow faster than another. Such a response produces definite, if relatively slow, movement. Two kinds of growth movements in response to outside stimuli are recognized in plants.

1. **Nastic movements.** A nastic movement (or *nasty* as it is sometimes called) is a response that is independent of the direction from which an external stimulus strikes the organism. The opening of certain flowers after sunrise is an example of a nastic movement. Illumination from any direction whatever will trigger the response, and the response is not oriented with respect to the direction of the stimulus. Although most nastic movements involve differential growth, that is, more rapid growth in certain parts than in others, some, such as the response of *Mimosa* leaflets, are turgor movements.

Positive vs. negative tropisms

2. **Tropisms.** A tropism is a growth movement whose direction is determined by the direction from which the stimulus strikes the plant. If the plant part grows in the direction from which the stimulus originates, the tropism is referred to as positive. Growth in the opposite direction constitutes a negative tropism.

It has been known for years that plants respond to both the stimulus of light and the stimulus of gravity. The former response is a **phototropism,** the latter a **geotropism.** Stems exhibit positive phototropism while roots are negatively phototropic. On the other hand, stems are negatively geotropic while roots are positively geotropic (Fig. 24.2). The value to the plant of these responses seems quite clear. Roots growing downwards and/or away from light are more likely to find soil, water, and minerals. Stems growing upwards or towards light will be able to expose their leaves so that photosynthesis can occur.

In addition to growth movements, we know that several important *developmental* changes in plants occur in response to environmental stimuli.

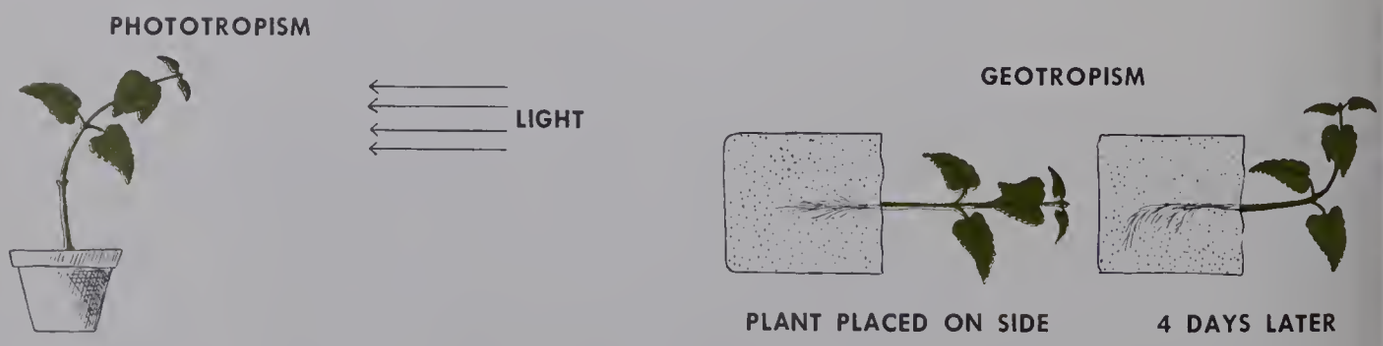


Fig. 24.2 Tropisms. The direction of plant growth is affected by light (phototropism) and gravity (geotropism).

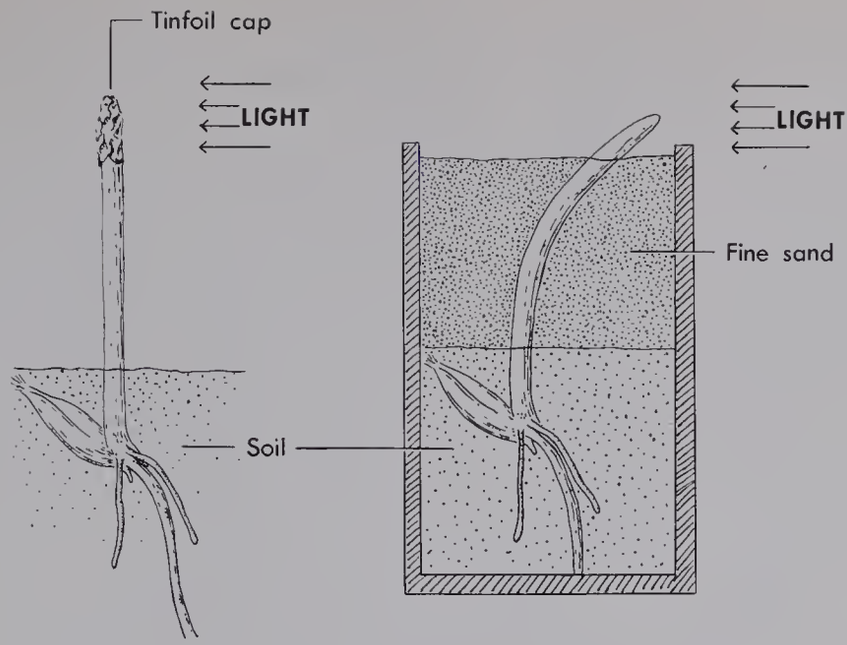


Fig. 24.3 The Darwins' experiments which showed that the response of phototropism is dependent upon light striking the tip of the plant.

Developmental changes in response to environmental stimuli

The germination of seeds, the resumption of growth of perennials in the spring, and the development of flowers all come about as a result of environmental triggers. The problem of understanding how plants respond to changes in their environment is twofold. First, we must discover how the plant detects the specific stimulus. Second, we must discover how the various tissues of the plant are coordinated in carrying out the response.

24.3 THE MECHANISM OF PHOTOTROPISM

The Darwins demonstrated that the tip of the plant detects the direction of light

The first clues to the coordinating mechanisms in plants came through a study of phototropism reported by Charles Darwin and his son Francis in 1880. They discovered that when the tip is removed from a growing shoot, the shoot ceases to exhibit phototropism. This was particularly surprising in view of their additional discovery: the bending action of the plant stem occurs in the region *behind* its tip. They found that if they placed an opaque covering over the tip of the plant, phototropism failed to occur even though the rest of the shoot was illuminated from one side. On the other hand, when they buried a plant in fine black sand so that only the tip of the shoot was exposed, there was no interference with phototropism. When the tip was illuminated from the side, the buried stem promptly bent in the direction of the light (Fig. 24.3). From these experiments, it seemed quite clear that the stimulus (light) was detected at one location (the tip) and the response (bending) was carried out at another (the region of elongation). This implied that the tip was, in some way, communicating with the cells of the region of elongation.

The Darwins confirmed these results with several different plant species. They found that grass seedlings were particularly easy subjects for this kind of experimentation. When grass seeds germinate, the primary leaf of the plant pierces the seed coverings and soil layers. It is protected from damage as it does so by a hollow, cylindrical structure, the **coleoptile,**

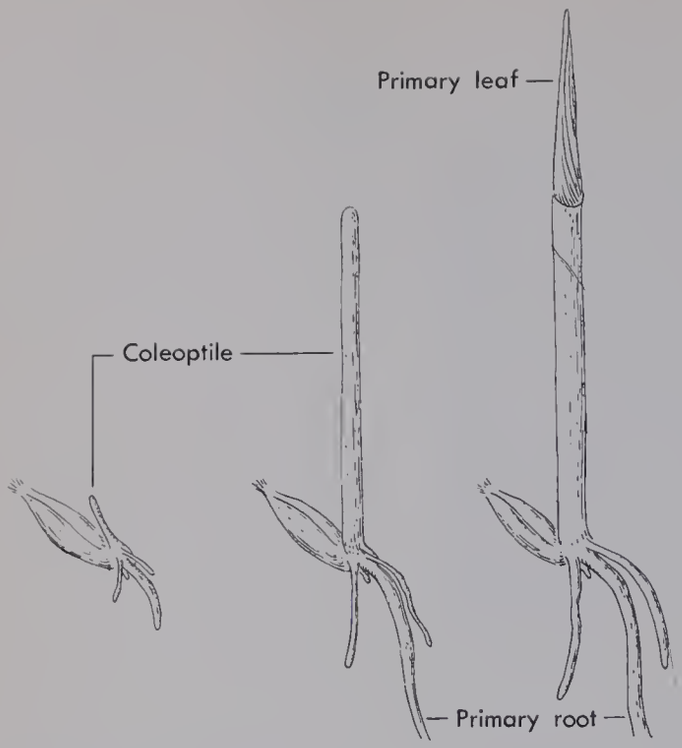


Fig. 24.4 Stages in the germination of an oat *(Avena)* seed.

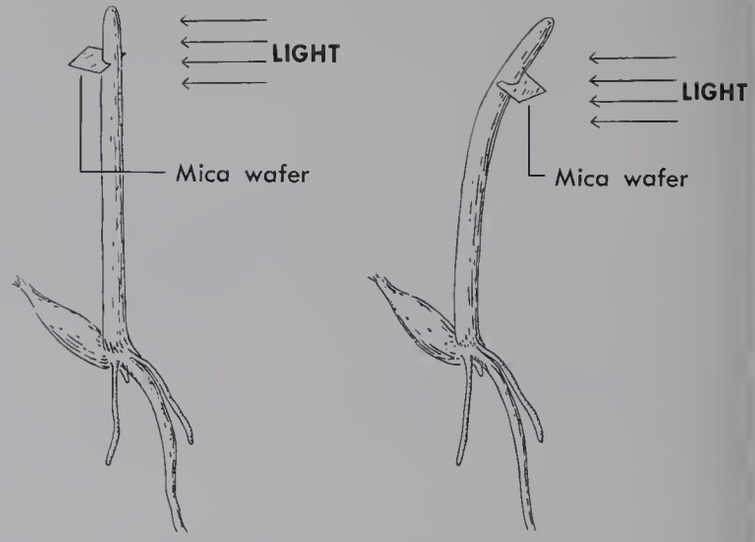


Fig. 24.5 Boysen-Jensen's experiments. The wafer of mica interfered with the phototropic response only when inserted on the shady side.

The coleoptile is a protective sheath found in grass seedlings

which surrounds it (Fig. 24.4). Once the seedling has grown up above the surface of the ground, growth of the coleoptile ceases and the primary leaf pierces it.

The Darwins found that the tip of the coleoptile was necessary for phototropism in grass seedlings just as the shoot tip is necessary in older seedlings or in dicots such as the bean. Also, they found that the actual bending of the coleoptile occurs in the region below the tip. Thus a system of communication seems to be involved here, too.

The experiments of Boysen-Jensen

In 1913, the Danish plant physiologist Boysen-Jensen showed that this communication must be accomplished by means of a chemical substance passing downward from the tip of the coleoptile. He cut off the tip of the coleoptile, covered the stump with a layer of gelatin, and then replaced the tip. Phototropism was still accomplished successfully. However, a piece of impervious material, such as mica, placed between the tip and the stump prevented the phototropic response. Interestingly enough, this interference occurred only when the sheet of mica separated the tip and stump on the shady side of the plant. When a horizontal incision was made in the illuminated side and the mica inserted, no interference with phototropism occurred. This suggested that the chemical coordinator was passing down the dark side of the seedling only. It also suggested that the chemical coordinator was a growth stimulator, as the phototropic response involves faster cell elongation on the shady side than on the sunny side (Fig. 24.5).

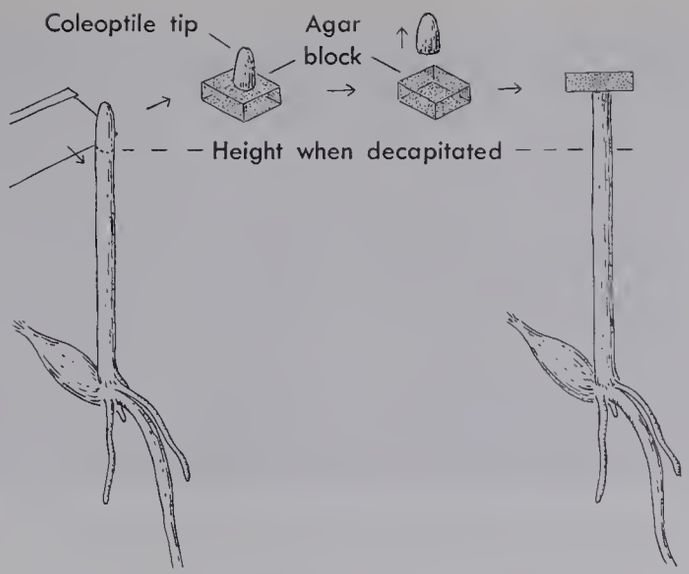


Fig. 24.6 Went's experiment showed that a chemical growth stimulator —auxin—can be extracted from coleoptile tips and still retain its effectiveness. Several tips should be placed on the agar block for a good response.

24.4 THE DISCOVERY AND ROLE OF AUXIN

Went first extracted auxin

F. W. Went first extracted this growth stimulator. He removed the tips from several coleoptiles of the oat plant (a grass), *Avena sativa*. He placed these tips on a block of agar and let them remain there for several hours. At the end of this time, the agar block itself was able to initiate resumption of growth in a decapitated coleoptile (Fig. 24.6). The growth was vertical because the agar block was placed completely across the stump of the coleoptile, and no light reached the plant from the side. This experiment showed that a chemical growth stimulator had diffused from the tips into the agar block. The name **auxin** was given to this material.

Unfortunately the quantity of active material in the coleoptile tips was far too small to be purified and analyzed chemically. Therefore, a search was made for other sources of auxin. In this search, research workers were greatly aided by a technique, developed by Went, for determining the relative amount of auxin activity in a given preparation. The material in question is incorporated into an agar block and the block is placed on one side of a decapitated coleoptile (Fig. 24.7). As the auxin diffuses into that side of the coleoptile, it stimulates cell elongation and the coleoptile bends

Measuring auxin activity with a bioassay

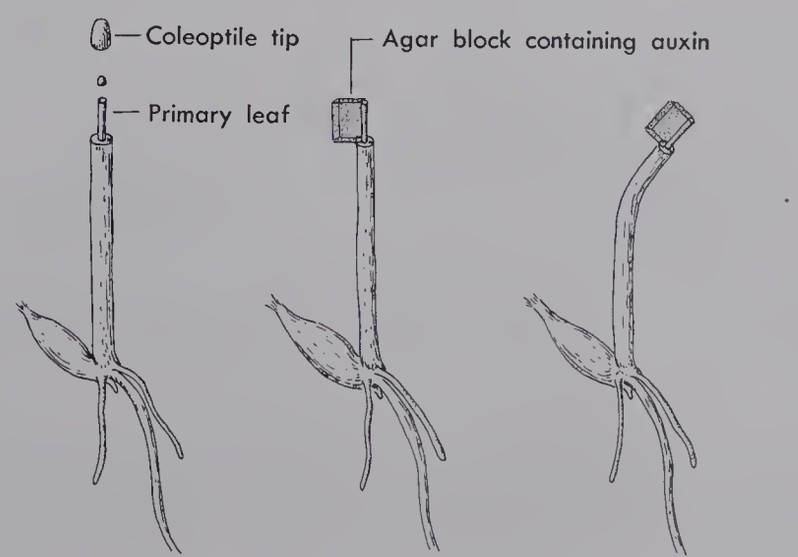


Fig. 24.7 The *Avena* test. The degree of bending is proportional to the amount of auxin activity in the agar block. The use of a living organism to determine the amount of activity of a substance is called a bioassay.



Fig. 24.8 Photographic record of an *Avena* test. (Courtesy of Dr. Kenneth V. Thimann.)

away from the block. The degree of curvature, measured at the end of 1½ hours, is proportional to the amount of auxin activity (e.g. number of coleoptile tips used) in the agar block. The use of a living organism to determine the amount of a given substance present is called a **bioassay** (Fig. 24.8).

Using Went's technique, it was soon found that auxins occur widely in nature. One of the most potent auxins was actually first isolated from human urine. It is indoleacetic acid or IAA. Although it is only one of many growth-promoting substances which have been discovered, there is good evidence that it is the most important auxin produced by plants.

Auxin and phototropism

To Went also goes the credit for discovering that an unequal distribution of auxin is responsible for the bending in phototropism. When a coleoptile tip that has previously been illuminated from one side is placed on two separated agar blocks (Fig. 24.9), the agar block on the side that had been shaded accumulates almost twice as much auxin as the block on the previously lighted side. This explains why cell elongation occurs more

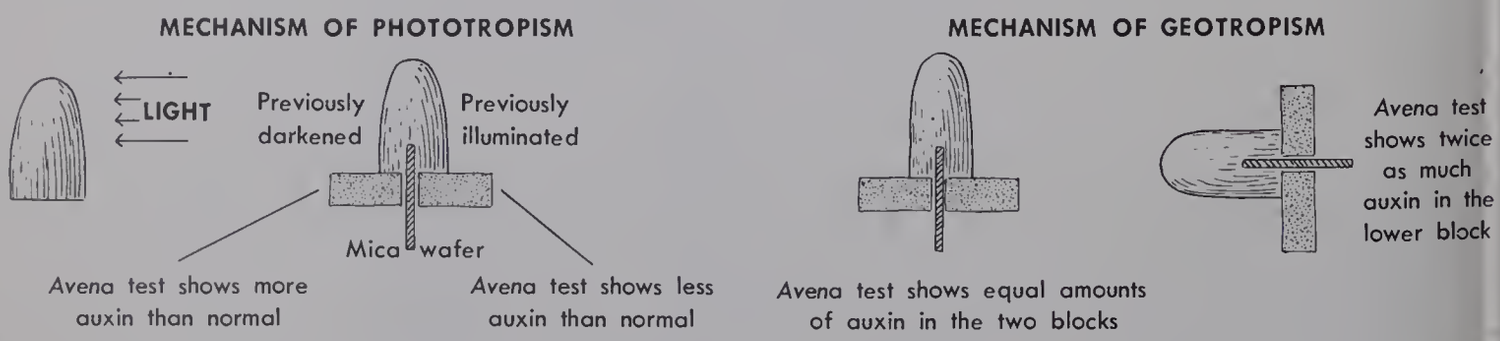


Fig. 24.9 Unequal distribution of auxin in phototropism and geotropism. In the intact plant, the side of the coleoptile receiving the greater concentration of auxin would grow more rapidly, resulting in bending toward light and away from the force of gravity.

rapidly on the shady side of the plant. Further investigation has demonstrated that light causes auxin to be translocated to the shady side.

Auxin and geotropism

Unequal distribution of auxin also explains the phenomenon of geotropism. When an oat coleoptile tip is placed on two separated agar blocks, as shown on the left in Fig. 24.9, there is no difference in the auxin activity picked up by the two blocks. When the two agar blocks are applied to a coleoptile tip placed on its side, however, the lower block accumulates twice as much auxin activity as the upper block. Under natural circumstances, this would result in greater cell elongation on the underside of the coleoptile. Thus the plant would bend upwards (Fig. 24.2).

Roots are inhibited by concentrations of auxin that stimulate stems

How can one explain the negative phototropism and positive geotropism of *roots?* It seems as though exactly the same mechanism is at work in roots as in stems. The difference lies in the sensitivity of the cells of the root as compared with those of the stem (Fig. 24.10). Concentrations of auxin that stimulate growth in stems actually inhibit growth in roots. Concentrations of auxin which are too low to have any effect upon stems give some stimulation of cell elongation in the root. Therefore it is the illuminated side and the upper side of the root that grow more rapidly. As a result the root bends away from the light and downwards.

Blue light is most effective in phototropism

Although we have discovered a mechanism that coordinates these plant responses, we do not yet know how the environmental stimuli are actually received. In the case of phototropism, we assume that some light-absorbing *pigment* must be involved. IAA itself cannot fill this role as it does not absorb visible light. Because the phototropic response is most sensitive to blue light, the pigment is probably yellow in color, most likely a carotenoid (see Section 9.2).

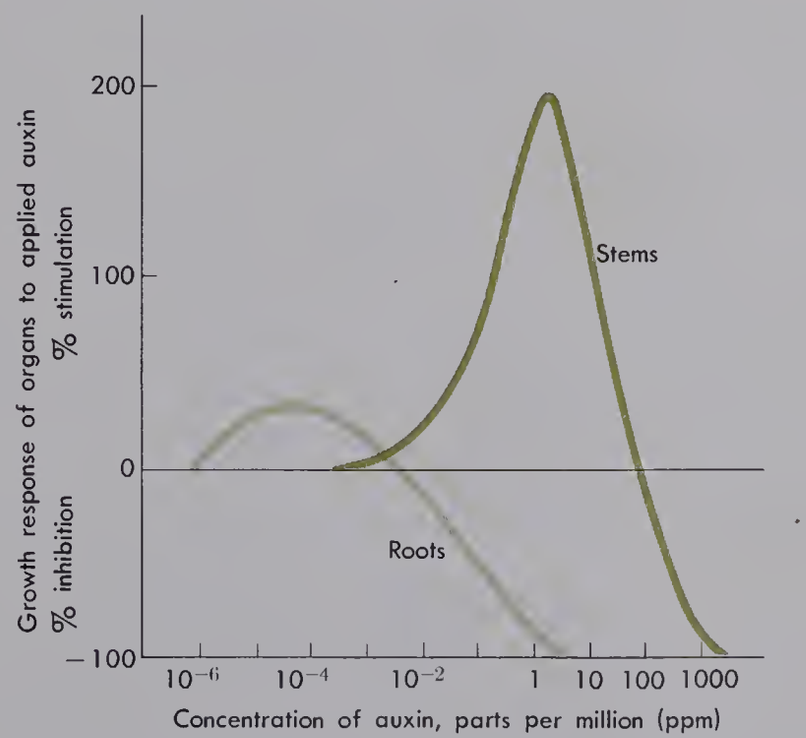


Fig. 24.10 The effect of auxin concentration on root and stem growth. Concentrations of auxin that stimulate stem growth inhibit the growth of roots. (Based on Fig. 2, K. V. Thimann, *Amer. Journal of Botany* **24,** 411, 1937.)

24.5 OTHER AUXIN ACTIVITIES

The discovery of auxins opened up a very active field of research. Over a brief period of time, auxins were found to participate in the coordination of a variety of other plant activities.

Developing seeds release auxin

1. Fruit development. Pollination of the flowers of angiosperms initiates the formation of seeds. Each seed contains the embryo of a new plant. As the seeds mature, surrounding flower parts form a covering, the fruit, around the seeds. It is now known that as the seeds develop, they release auxin into the surrounding flower parts, thus stimulating the growth of the fruit. In fact, it is possible to stimulate fruit development in nonpollinated flowers simply by the application of auxin to the flower. This is not just a laboratory curiosity. Many tomato and holly growers deliberately initiate fruit development in this way. Not only does this ensure that all flowers will "set" fruit, but it also increases the likelihood that all the fruits will be ready for harvesting at the same time.

The terminal shoot inhibits the development of lateral buds

2. Apical dominance. As a general rule, growth of the shoot apex (terminal shoot) of a plant inhibits the development of the lateral buds on the stem beneath. This inhibition is called apical dominance. In trees which form a single, straight trunk (e.g. most pines), apical dominance is very pronounced. In low-growing, many-branched, shrubby plants, it is less noticeable. If the terminal shoot is removed, the inhibition is removed with it (Fig. 24.11). The lateral buds then commence growth. Gardeners regu-

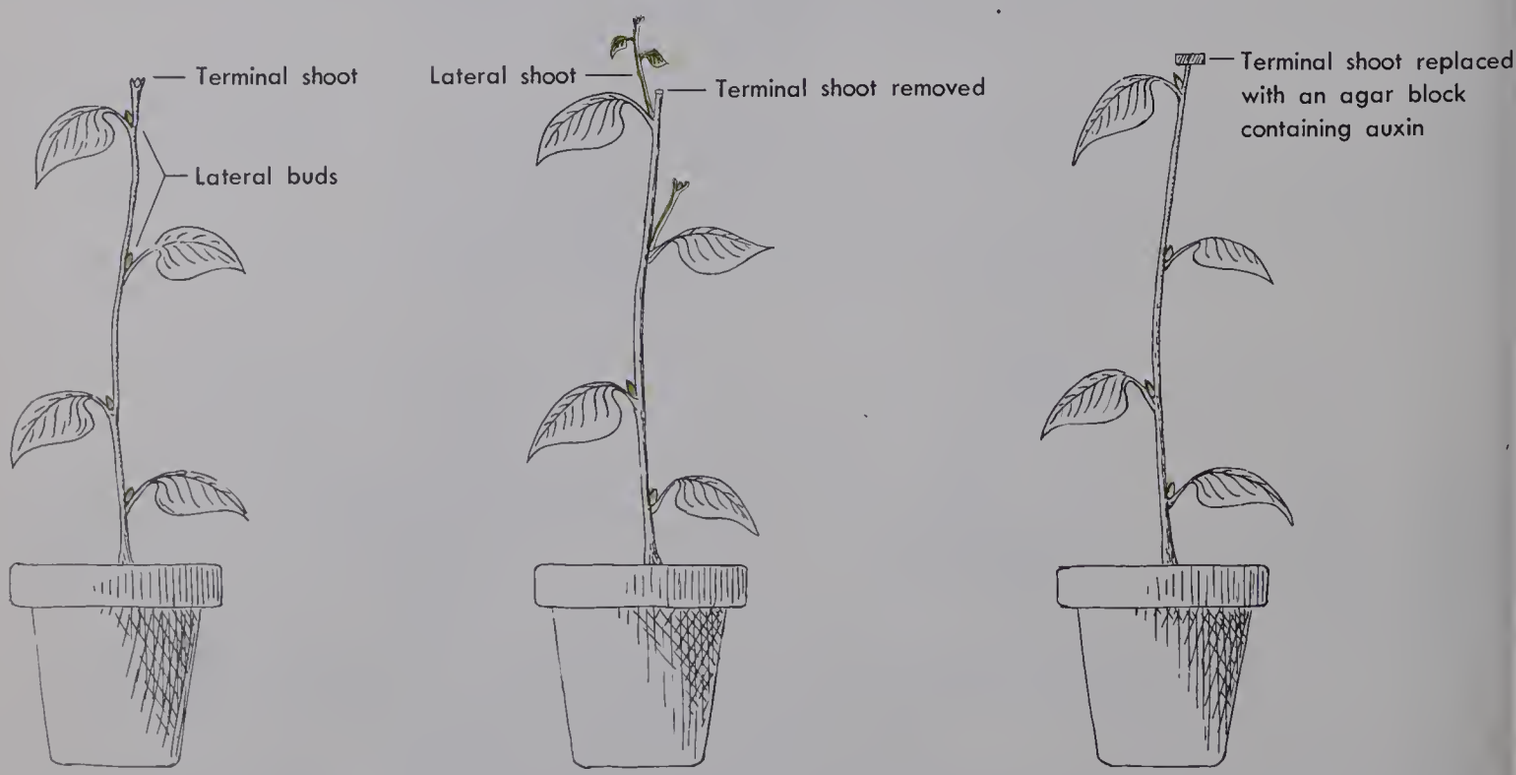


Fig. 24.11 Apical dominance. Translocation of auxin from the terminal shoot (left) or an auxin-containing agar block (right) inhibits development of the lateral buds. Absence of a source of auxin enables the lateral buds to develop (center).



Fig. 24.12 When the white pines in this grove were young, their terminal shoots ("leaders") were killed by the white pine weevil. With the removal of apical dominance, two lateral branches succeeded in replacing the leader on the tree the boys are standing near. (Courtesy of Owen MacNutt.)

larly exploit this principle by pruning the terminal shoot of ornamental shrubs. The release of apical dominance enables lateral branches to develop and the shrub becomes fuller and less spindly. The process must be repeated periodically, though, because one or two of the "laterals" will eventually outstrip the others and reimpose apical dominance.

Apical dominance seems to result from the downward transport of auxin produced in the apical meristem. In fact, if the apical meristem is removed and a block of auxin-containing agar placed on the stump, inhibition of the lateral buds is maintained (Fig. 24.11). A plain block of agar has no such inhibiting effect.

White pines grown in warm, sunny locations frequently are parasitized by the white pine weevil. This insect lays eggs in the terminal shoot of the tree. After the young hatch, they consume the tissues of the shoot and kill it. Death of the shoot removes apical dominance and the lateral branches beneath begin to grow rapidly upward. If left alone, one or two laterals usually succeed in reestablishing apical dominance. Figure 24.12 shows the crotch formed in a white pine by the replacement of one terminal shoot by two.

The principle of apical dominance can easily be demonstrated in the laboratory. The common white potato is really a portion of the underground stem of the potato plant. It has a terminal (apical) bud or "eye" and several lateral buds. After a long period of storage, the terminal bud usually sprouts but the other buds do not. However, if the potato is sliced into sections, one bud to a section, the lateral buds develop just as quickly as the terminal bud (Fig. 24.13).

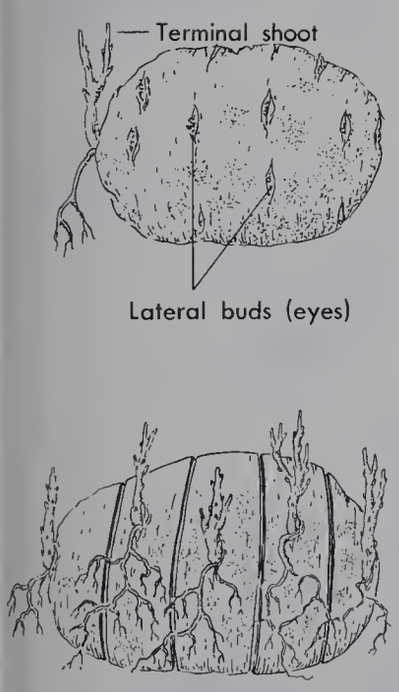


Fig. 24.13 Apical dominance in the white potato. In what ways is this demonstration similar to that shown in Fig. 24.11?

Abscission is the shedding of leaves and fruit

3. Abscission. It has been found that auxins play an important role in the shedding of leaves and fruit. Young leaves and fruits produce auxin and as long as they do, they remain firmly attached to the stem. When auxin production diminishes, however, a special layer of cells forms at the base of the petiole or fruit stalk. This layer of cells is called the abscission layer (Fig. 24.14). Soon the petiole or fruit stalk breaks free at this point and the leaf or fruit falls to the ground.

One can demonstrate this nicely in the laboratory. If the blade of a coleus leaf is removed, as shown in Fig. 24.15, the petiole remains attached to the stem for just a few more days. The removal of the blade seems to be the trigger here, as an undamaged leaf at the same node of the stem remains on the plant much longer, in fact, the normal length of time. If, however, auxin is applied to the cut end of the petiole (Fig. 24.15), abscission of the petiole is greatly delayed.

Auxin inhibits abscission

The ability of auxin to delay abscission has been exploited by apple and orange growers. The fruits of these species often drop off before they are ready for picking. This may result in a severe financial loss to the grower. Carefully applied auxin sprays greatly cut down loss by premature dropping.

Just what causes the lowered production of auxin in mature leaves and fruits is not completely clear. Leaves which are entirely shaded by others above them soon cease to produce auxin and drop off. This is a valuable

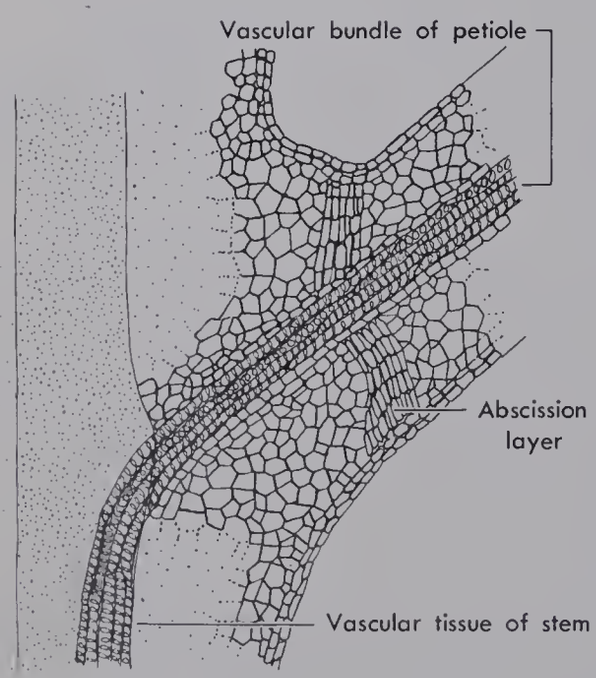


Fig. 24.14 The abscission layer. When leaves and fruits fall from a plant, the separation occurs at the abscission layer.

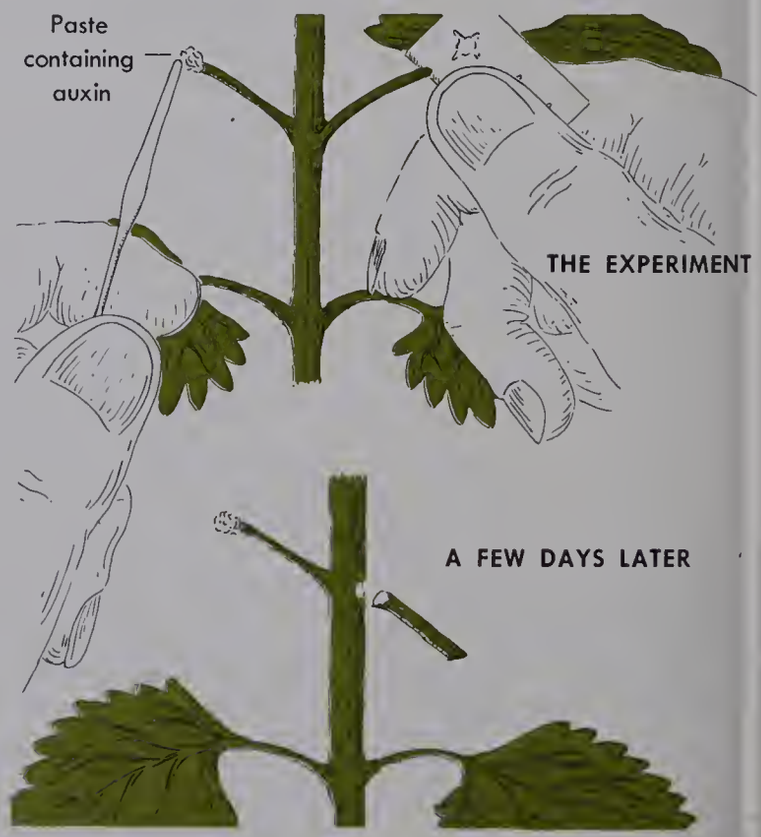


Fig. 24.15 The role of auxin in abscission. The petiole of a leaf whose blade has been removed soon drops from the stem unless auxin is applied to the cut surface.

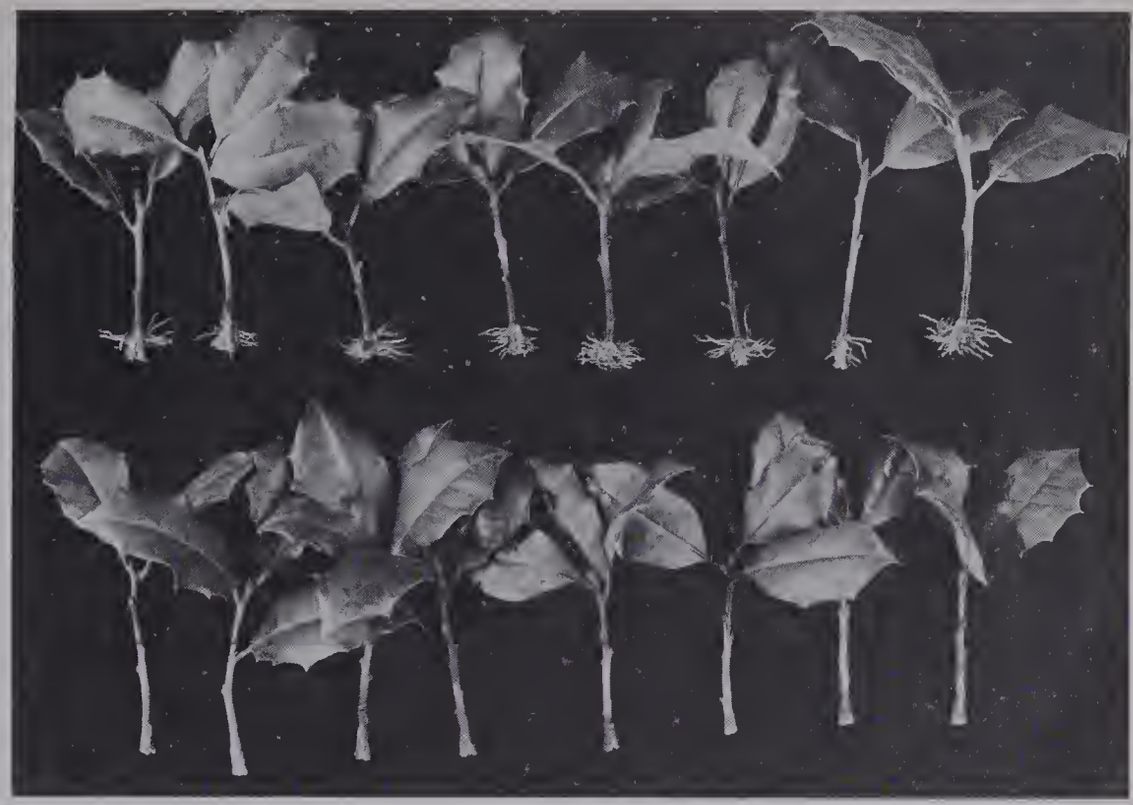


Fig. 24.16 Top: American holly cuttings which have been treated with a synthetic auxin (beta-indole-butyric acid). Bottom: untreated controls. (Courtesy of Paul C. Marth, U.S.D.A., Beltsville.)

adaptation of the plant because a shaded leaf needs nourishment but cannot manufacture it by photosynthesis. Thus it becomes a detriment to the plant.

Importance of leaf drop

In the fall, all the leaves of deciduous trees drop off. This, too, is a valuable adaptation because it reduces the surface area of the plant, thus diminishing water loss and snow load. As far as the plant's metabolism is concerned, freezing weather is equivalent to a drought. Leaf drop thus serves to conserve water and to minimize snow damage during the winter. (Most nondeciduous, cold-climate species have "needles" for leaves. These are very narrow and have a heavy, waterproof cuticle. The shape aids in the shedding of snow, and the cuticle cuts down on water loss.)

Adventitious roots develop from stems or leaves

4. Root initiation. Auxins also stimulate the formation of **adventitious roots** in many species. Adventitious roots arise from stems or leaves rather than the regular root system of the plant. Gardeners may propagate desirable plants by cutting pieces of stem and placing them base down in moist sand. Eventually, adventitious roots grow out at the base of the cutting. Their development seems to depend, among other things, upon the presence of auxin which has been translocated down from the upper portions of the cutting. Many gardeners now hasten the process by pretreating their cuttings with solutions or powders containing synthetic auxins (Fig. 24.16).

24.6 HOW DO AUXINS WORK? A THEORY

As we have seen, auxins affect a variety of plant tissues in a variety of different ways. Some tissues (e.g. coleoptile, ovary wall of the pistil) are stimulated by auxin. Some (e.g. lateral buds) are inhibited. How can a single small molecule of this sort produce such a variety of effects?

The answer is not yet known with certainty. However, at least some of the effects of auxin on plant cells can be traced to its influence on the genes themselves. The stimulating effect of auxin on coleoptile and stem growth has been shown to be strongly inhibited by an antibiotic called **actinomycin D.** Actinomycin D exerts its lethal effect on cells in a remarkably precise way. It binds to the DNA molecules in the nucleus of the cell and prevents the two strands of DNA from separating. Thus the DNA molecule cannot serve as the template for the synthesis of either additional DNA (Fig. 19.5) or RNA molecules (Fig. 19.16). Without fresh supplies of RNA, the synthesis of new proteins (including all enzymes) by the cell soon comes to a halt. The inhibition of auxin action by actinomycin D thus suggests that at least one effect of auxin is on the transcription of the DNA code in the cell. This has been further verified by the discovery that when isolated plant nuclei are treated with IAA, there soon follows a marked increase in the production of RNA. However, when actinomycin D is added to the system, this increase is prevented.

Actinomycin D blocks the transcription of DNA

Treatment of plant cells with auxin produces an increase not only in RNA production but in protein production as well. This is to be expected in view of the role that messenger, ribosomal, and transfer RNA molecules play in protein synthesis (see Section 19.5). At least one specific protein, an enzyme, has been found to be induced in pea stems exposed to auxin. This induction, too, is blocked by actinomycin D, and therefore we may conclude that auxin has an effect on the genetic code itself. A specific gene coding for a specific enzyme has been unlocked by the presence of IAA.

Auxin enhances transcription of the genetic code

It is not yet known exactly how auxin interacts with the genetic code to unlock genes. There is evidence, however, that auxin is active within the cell only when joined to a protein molecule. The resulting complex appears to increase the activity of RNA polymerase and, as a result, leads to the production of more (and perhaps different) RNA molecules (i.e. new messengers).

The foregoing discussion should not obscure the fact that *some* of the varied activities of auxin occur too rapidly to be a result of gene activation. Auxin must influence cell activities in other, more rapid, ways as well. Only further experimentation will show how these effects are achieved.

24.7 THE GIBBERELLINS

Other growth-promoting substances have been discovered in addition to the auxins. During the 1930's, Japanese scientists isolated such a substance from cultures of a fungus which parasitizes rice plants. They called this substance gibberellin. After the delay caused by World War II, plant

physiologists in other countries took up the trail and succeeded in isolating several more closely related compounds. These are now known collectively as gibberellins.

The effect of gibberellins on stem growth

Perhaps the most dramatic effect of the gibberellins is on stem growth. When applied in low concentrations to a bush or "dwarf" bean, the stem begins to grow very rapidly. The length of the internodes becomes sufficiently great that the plant becomes indistinguishable from climbing or "pole" beans. Gibberellin seems to overcome the hereditary limitations in many "dwarf" plant types.

Although gibberellins somewhat resemble auxins in their stimulation of stem growth, they are not classified as auxins. Not only is their molecular structure quite different from that of the auxins, but they do not give a response in the oat coleoptile test.

The gibberellins (like the auxins) were first isolated from sources other than the higher plants themselves. This, of course, raises the question of whether they are normal constituents of the higher plants and, if so, what role they play in the normal coordinating mechanisms of the plant. In recent years gibberellins have indeed been found to occur naturally in a variety of plant tissues. In addition to being implicated in the growth of stems, they seem to be the chief stimulants of root growth. The application of gibberellins to certain plants (e.g. spinach, cabbage) promotes the development of flowers. That this reflects a natural activity is suggested by the presence of increased quantities of gibberellins in the tissues of these plants when they are normally ready to flower.

Effect on sprouting of buds

Gibberellins also appear to play a role in the sprouting of buds. When white potatoes are first harvested, neither the terminal nor the lateral buds will sprout (Fig. 24.13). However, the application of gibberellin to the terminal bud will cause it to sprout immediately. In such woody species as the peach, birch, and sycamore, the synthesis of gibberellins in the spring seems to be the trigger for the sprouting of buds, which have been dormant during the cold winter months.

Effect on seed germination

The germination of seeds is another stage of plant life in which gibberellins may play a major role. You remember that most of the food reserves of cereal grains such as barley, wheat, rice, and corn are stored in the endosperm (Fig. 20.9). One of the first steps in the germination process in these monocots is production of gibberellin by the embryo. The gibberellin acts on the cells surrounding the endosperm, causing them to produce a number of specific hydrolytic enzymes (e.g. amylase) that digest the starch and protein of the endosperm and thus make sugars and amino acids available to the growing embryo. These enzymes also break down the seed coats and thus make it easier for the radicle and coleoptile to break through them (Fig. 24.4). This effect of gibberellin can be blocked by actinomycin D, a fact suggesting that the gibberellin is activating genes within the cells surrounding the endosperm. In fact the application of synthetic gibberellin to these cells first produces a burst of RNA synthesis. This is followed by the synthesis of the various hydrolytic enzymes.

24.8 THE CYTOKININS

Another group of plant growth regulators are the cytokinins. Each of these substances contains the purine, adenine, as part of their molecular structure. (What other substances have you studied that contain adenine?)

Cytokinins stimulate mitosis

Cytokinins, when acting together with auxin, strongly stimulate mitosis in meristematic tissues. The food reserves of some seeds contain cytokinins. Presumably these provide the chemical stimulus for rapid mitosis in the developing seedling. Cytokinins also promote the differentiation of the cells produced in the meristems.

In addition to their growth-promoting effects, cytokinins have been shown to slow down the aging of plant parts, such as leaves, and to increase the resistance of plant parts to such harmful influences as low temperatures, virus infection, weed killers, and radiation.

As is the case with auxins and gibberellins, at least some of the effects of cytokinins are brought about by selective gene activation. Two enzymes have been shown to be specifically induced by cytokinins and this induction is blocked by actinomycin D. Furthermore, a marked burst of RNA (probably messenger RNA) synthesis occurs when plant cells or isolated nuclei are treated with cytokinins.

24.9 ABSCISIC ACID

Inhibitors also play a role in the coordination of plant activities. In the fall, the mature leaves of such trees as the birch and sycamore produce a substance which stops growth in the apical meristems of the stems and converts them into dormant buds. The newly developing leaves growing above the meristem (look back at Fig. 23.2) become converted into stiff bud scales that wrap the meristem closely and will protect it from mechanical damage and drying out during the winter months. The substance responsible for the conversion of the apical meristems into dormant buds has been identified and named abscisic acid. Presumably it passes from its place of manufacture, the mature leaves of the plant, to the apical meristems by way of the phloem.

Formation of dormant buds

Breaking bud dormancy

Once a bud has become dormant, it usually cannot be reactivated simply by the return of warm temperatures and abundant moisture. In some cases, gibberellin synthesis is necessary (probably triggered by the lengthening days of spring). In some cases, a period of exposure to cold temperatures is necessary before bud dormancy can be broken. Apple trees and lilacs, for example, cannot be grown in tropical areas because the winters never get cold enough (a few days at 0–10°C) to break bud dormancy.

The value of enforced dormancy to the plant is quite clear. If inhibitors such as abscisic acid were not present in the late fall and early winter, an unexpected warm spell might stimulate bud sprouting. The return of freezing weather would quickly kill this delicate growth. Enforced dormancy, which can either be ended after a period of prolonged cold or with

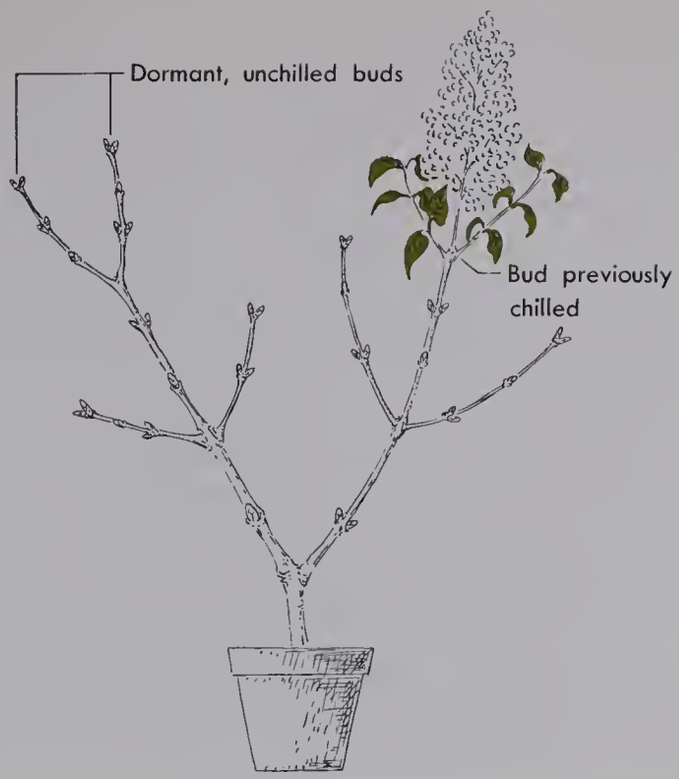


Fig. 24.17 Breaking bud dormancy in the lilac by localized cold treatment. Even under the best growing conditions, lilac buds will not develop without prior chilling.

the return of longer days, prevents the plant from being endangered in this way.

The dormancy of buds seems to be a very localized thing. By prior chilling, one bud on a lilac stem can be stimulated to grow while the other, nonchilled buds on the stem remain quite dormant (Fig. 24.17).

Breaking seed dormancy

Many newly-formed seeds must also undergo a period of enforced dormancy before they can germinate. Abscisic acid may be involved in some cases (e.g. in peach seeds) but other inhibitors have been discovered, too. Exposure to cold temperatures or, in some cases, exposure to sufficient amounts of water to leach the inhibitor out of the seed, is necessary before germination can occur. The value to the seed of a cold requirement is the same as for buds. As for a water requirement—beyond that needed for the process of germination itself—you remember (see Section 20.9) that this ensures that the seeds of desert plants will not germinate if there has not been sufficient rainfall to enable them to complete their entire life cycle.

Abscisic acid also has been found to speed up the abscission of aging plant parts such as leaves and fruits (Fig. 24.14). It was this effect, which was discovered independently of its effect on dormancy, that gave rise to the name.

In contrast to auxins, gibberellins, and cytokinins, abscisic acid appears to be a repressor of gene activity. It inhibits the synthesis of RNA (also DNA). However, this inhibition can be reversed by the presence of cytokinins.

24.10 ETHYLENE

Ethylene hastens the ripening of fruit

When first harvested, lemons are often too green to be acceptable in the market. In order to hasten the development of a uniform yellow color, lemon growers used to store the newly harvested lemons in sheds which were kept warm and humid. The warmth was supplied by kerosene stoves. When one grower tried a more modern heating system, he found that his lemons no longer changed color properly. Following this clue, it was soon found that the important factor in the ripening process was not the heat, but the small amounts of ethylene gas (CH_2CH_2) given off by the burning kerosene.

Since that time it has been found that most fruits produce their own ethylene, and it is this which triggers the ripening process. Among the many changes that ethylene brings about is a change in the permeability of the membranes of the cell. One consequence of this is to allow a chlorophyll-destroying enzyme into the chloroplasts. With the breakdown of chlorophyll, the red and/or yellow pigments in the cells of the fruit are unmasked and the fruit assumes its ripened color.

THE FLOWERING PROCESS

Flower buds are made from meristems

One of the most important developmental activities of angiosperms is flowering. When a plant is in active growth, mitosis in the apical meristem produces cells which go on to form more stem tissues and cells which will form leaf buds (Fig. 23.2). The leaf buds grow into mature leaves. Eventually, though, a time comes (usually as active growth ceases) when the meristems form flower primordia. These are clusters of cells which develop into flower buds. The flowers, into which these buds mature, contain the sex organs of the angiosperms, without which sexual reproduction could not occur.

24.11 FACTORS THAT INITIATE FLOWERING

The importance of the flowering process has led many plant physiologists to try to find out what initiates it. The question is simply, "What causes the plant to stop producing leaf buds and start producing flower buds?"

Importance of adequate food reserves

In some cases, the stimulus seems to be purely internal. Certain tomato varieties automatically produce flower primordia after 13 nodes have been produced by the growing stem. Adequate food reserves must be present in the plant, however. If the plant has not been photosynthesizing actively, it will lack the energy reserves necessary for the flowering process.

In most cases, the stimulus which initiates the flowering process seems to be external. Temperature often serves as the critical stimulus. This is particularly true of biennial species, that is, plants that require two grow-

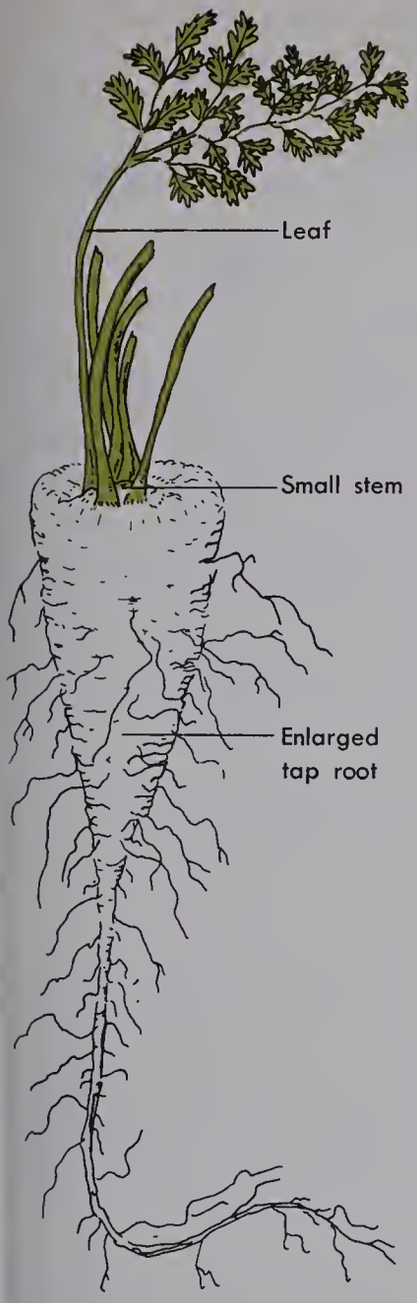


Fig. 24.18 The carrot, a biennial. Flowering occurs during the second growing season.

ing seasons in order to complete their life cycle. Beets, carrots, and cabbages are three common biennial plants. In the first growing season, they develop roots, a short stem, and a cluster of leaves (Fig. 24.18). During this season, food is stored in the root system. With the arrival of cold weather, the tops die back. The next season, flowers are produced on the new shoot growth. After the reproductory process is completed, the entire plant dies. Flowering will not occur in the second season, however, unless the plant has been exposed to cold weather during the winter.

Experiments have shown that the entire plant need not be chilled for successful flowering to follow. The terminal bud is the temperature receptor. Its exposure to temperatures in the range of 1–10°C sets the stage for subsequent flowering. Gibberellins may be involved in the process. When gibberellin is applied to a terminal bud that has not been exposed to cold, the plant proceeds to flower normally. Furthermore, when a cold-treated biennial is grafted to a non-cold-treated one, the latter also flowers vigorously. This suggests that the chilling of the terminal bud is followed by the production and translocation of a flower-promoting substance.

Still another factor that triggers the flowering process in many plant species is a change in the daily intervals of illumination to which the plant is exposed. This discovery was made in 1920 by two plant physiologists, W. W. Garner and H. A. Allard, employed by the United States Department of Agriculture. They were perplexed to find that a new variety of tobacco, Maryland Mammoth, did not flower during the summer as other tobacco varieties do. Protected in a greenhouse, however, this variety did flower successfully near Christmastime. After considerable experimentation with artificial lighting in the winter and artificial darkening in the summer, they concluded that the flowering response was governed by the length of the day. They called this behavior **photoperiodism.** Because Maryland Mammoth would flower only when exposed to short periods of light, they called it a short-day plant. Other species such as chrysanthemums, poinsettias, and the cocklebur are also short-day plants. Some plant species, e.g. spinach, sugar beets, and the radish, flower only after exposure to long days and hence are called long-day plants. Still other species, such as the tomato, are day-neutral, that is, their flowering is not closely regulated by the length of their exposure to light.

The discovery of photoperiodism explained several puzzling facts about plant development. First, it provided a mechanism to explain, partly at least, the regular succession of blooms which occurs during the growing season. From the first flowers of early spring to the last flowers in the fall, one plant species after another flowers in a sequence as regular as clockwork. Now one could understand why spinach "bolts" and flowers during the summer months while chrysanthemums produce their blooms in the autumn. Second, photoperiodism explains why the plants of a given species usually bloom simultaneously. Though some individuals may have commenced growth earlier than others in the spring, in a given area they all bloom at the same time. Third, photoperiodism explains why many plant species can be grown successfully only in a rather narrow range of

latitude. Spinach, a long-day plant, cannot flower successfully in the tropics because the days never get long enough (14 hours). Ragweed, a short-day plant, fails to thrive in northern Maine because by the time the days become short enough (August) to initiate flowering, a killing frost is apt to occur before the reproductory process is completed and the seeds matured.

24.12 MECHANISM OF PHOTOPERIODISM

The discovery of photoperiodism stimulated a great many plant physiologists to explore the process further in an attempt to determine the mechanism of action. They soon found that the terms *short-day* and *long-day* were misnomers. Interrupting the daylight period with intervals of darkness had absolutely no effect on the flowering process. Interrupting the night period with artificial illumination was, however, quite a different story. When short-day plants like the cocklebur were illuminated even briefly at night, they failed to flower. Thus it became clear that it was not the length of day that was important to the flowering process, but the length of night. Short-day plants were really *long-night* plants. For example, a cocklebur, growing at the latitude of Michigan, will flower only after it has been kept in the dark for 8½ hours. If it is illuminated by a flash of light at any time during this period, it fails to flower.

Short-day plants require an uninterrupted period of darkness

Although the development of flower primordia occurs at the meristems, the photoperiodic stimulus is not detected there. The leaves are the receptors of the stimulus. If only one leaf, in fact just a small portion of one leaf, of the cocklebur is subjected to an 8½-hour period of darkness, the entire plant will flower even though all the rest of its leaves fail to receive a sufficiently long exposure to darkness (Fig. 24.19). This suggests that some stimulus, presumed to be a hormone, is passing from the leaf to the meristems.

Photoperiod is measured by the leaves

Grafting experiments lend solid support to this idea. If a cocklebur exposed to a favorable photoperiod is grafted to a cocklebur exposed to an unfavorable photoperiod, both plants will flower (Fig. 24.19). By grafting a series of plants together, it is even possible to estimate how rapidly the stimulus, which has been given the name **florigen,** is transported. Florigen is manufactured in the leaves and moves through the phloem to the meristems. Its arrival is marked by a number of biochemical changes including a burst of RNA synthesis. These are followed later by the morphological changes associated with the conversion of meristems into flower primordia. Although the molecular mechanisms at work are not yet known, the effect of the arrival of florigen seems to be the derepression of the genes controlling flowering.

Evidence for the existence of a flower-inducing hormone

In the cocklebur, at least, production of florigen is a one-time affair. Given one night of at least 8½ hours' duration, the plant will begin the flowering process even though it is subsequently placed back under unfavorable, short-night conditions.

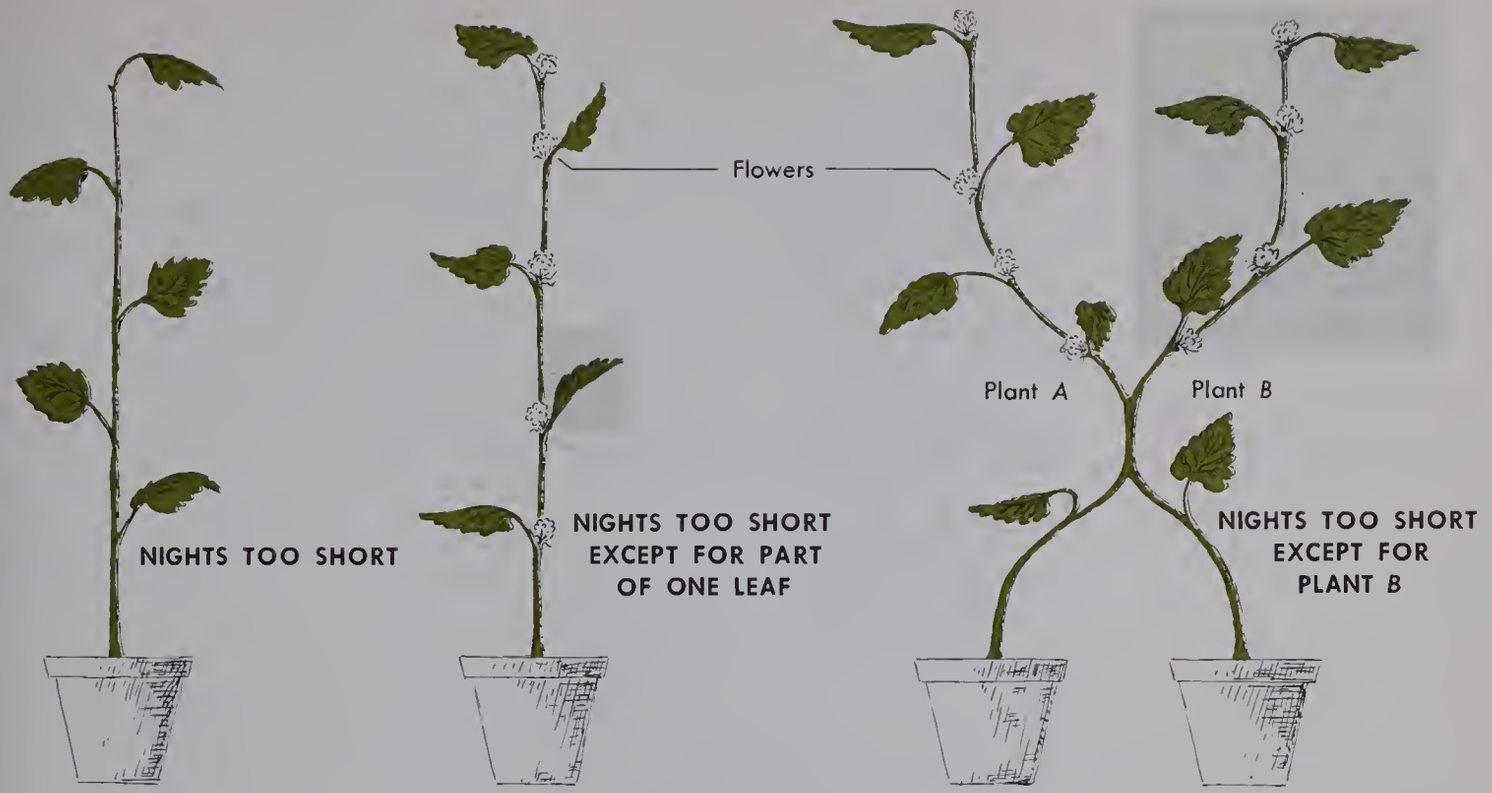


Fig. 24.19 Experimental evidence that the flowering stimulus is initiated in the leaf (center) and is transported by the vascular system to the flower buds (right).

The "long-day" plants are also misnamed. Spinach and other members of this group bloom successfully on a short-day schedule as long as the night periods are interrupted by a brief exposure to light. Thus, "long-day" plants are really *short-night* plants. They can bloom only if the nights are not too long.

Mechanism of photoperiodism in "long-day" plants

The mechanism of action in short-night plants seems somewhat more complicated than in long-night plants. When the short-night plant henbane is placed under long-night conditions, it fails to flower. If, however, some of its leaves are removed, or the plant is chilled, or it is placed in an anaerobic atmosphere during the long night, the inhibiting effect of the long night can be overcome and the plant does flower. This suggests that some inhibiting substance (it may be abscisic acid) is produced by the metabolism of the leaves during the hours of darkness. If too much of this substance accumulates, it inhibits flowering. Any interference, however, with the normal metabolism of the plant slows down the accumulation of this inhibitor.

On the other hand, there is evidence that a substance which *stimulates* flowering is released from leaves to meristems when the plant is under short-night conditions. In fact, this substance appears to be identical to the florigen produced by long-night plants. If a short-night plant is grafted to a long-night plant, both plants will flower under a short-night schedule, although the long-night plant would not normally do so.

24.13 THE DISCOVERY OF PHYTOCHROME

Photoperiodism must involve a very sensitive light-detecting mechanism. We have seen that the cocklebur fails to flower on a long-night schedule if the night is interrupted by even a brief flash of light. The light flash can be very dim, exposure to light not much brighter than the light of the full moon being effective.

The action spectrum of photoperiodism

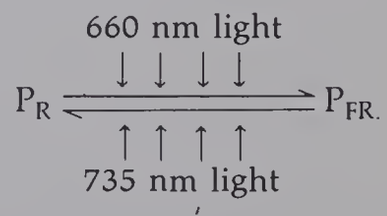
The most effective light rays for inhibiting flowering in the cocklebur are orange-red rays with a wavelength of 660 nm. The same wavelength is most effective in promoting flowering of spinach when its nights are otherwise too long. These findings suggest that the plants contain a pigment which absorbs orange-red light strongly.

It has also been found that the inhibiting effect of orange-red (660 nm) light on the cocklebur can be completely overcome by exposing the leaves of the plant to far-red light. This is light which is just beyond the visible spectrum. A wavelength of 735 nm is most effective in reversing the inhibitory action of orange-red light.

The system is completely reversible. The 8½ hour night of the cocklebur can be interrupted any number of times by orange-red light alternat- with far-red light. Whether flowering is inhibited or not depends upon the wavelength of the last flash. If the last flash is orange-red light, flowering will not occur. If it is far-red light, flowering will occur normally even though the plant has been exposed to substantial quantities of orange-red light prior to the final exposure (Fig. 24.20).

Phytochrome exists in two interconvertible forms

This reversible effect suggests the presence of a reversible pigment. In one condition, the pigment absorbs orange-red light. In so doing, it becomes converted to a form which absorbs far-red light. Exposure of the far-red absorbing form to far-red light reconverts the pigment to the orange-red absorbing form. The pigment has been given the name **phytochrome.** The orange-red-absorbing form is P_R. The far-red-absorbing form is P_{FR}. The two forms are interconvertible according to this scheme:



Although present in exceedingly small amounts, phytochrome has been isolated from plant tissue. It is a protein to which is attached a prosthetic group which gives it its light-absorbing property. As one could guess from the fact that phytochrome absorbs most strongly in the red end of the light spectrum, its color is blue.

The action of phytochrome in controlling photoperiodism seems to hinge on two factors: (1) Sunlight is richer in orange-red light (660 nm) than in far-red (735 nm) light. This means that at nightfall, all the phyto-

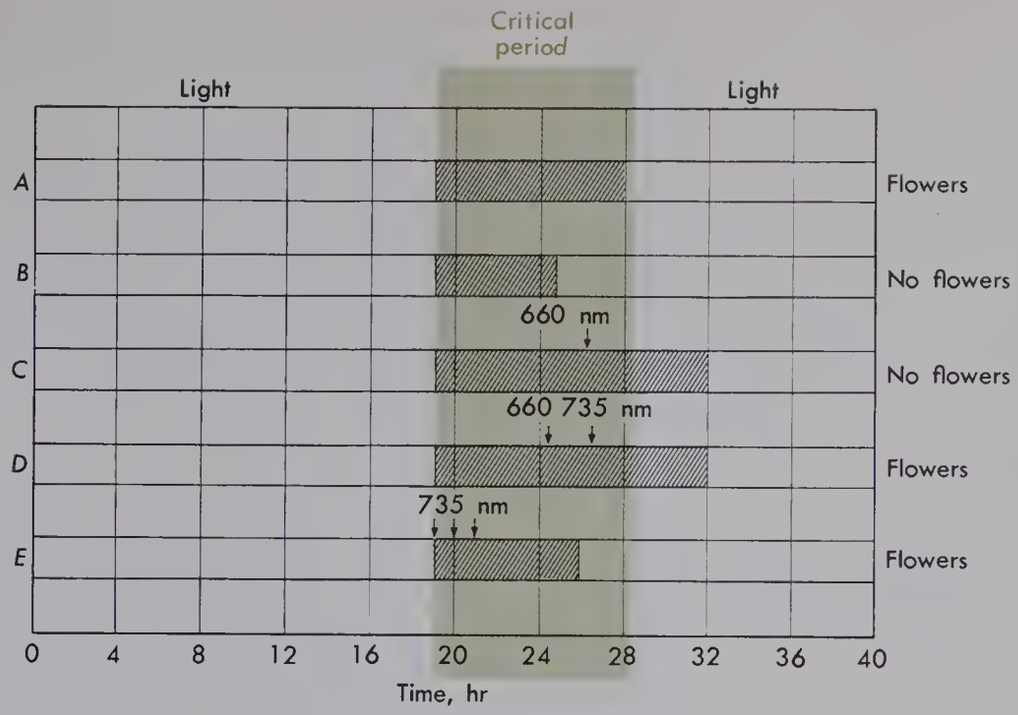


Fig. 24.20 Photoperiodism in the cocklebur. An uninterrupted night at least 8½ hours long is needed for flowering to occur (*A* and *B*). Interruption of an otherwise long night by orange-red (660 nm) light prevents flowering (*C*) unless it is followed by irradiation with far-red (735 nm) light (*D*). An intense exposure to far-red light at the start of the night reduces the dark requirement by two hours (*E*).

P_{FR} changes spontaneously into P_R

chrome in the leaves of a plant is in the form of P_{FR}. (2) P_{FR} is unstable while P_R is stable. In the darkness, P_{FR} gradually becomes reconverted into P_R. This conversion thus serves as the clock by means of which the plant measures the length of the night. In fact, it is possible to induce the cocklebur to flower after a night as short as 6½ hours if it is first exposed to a large dose of far-red light (Fig. 24.20). Presumably the far-red light accomplishes immediately the P_{FR} to P_R transition, which would otherwise require two or more hours of darkness. That a large dose of far-red light does not do away entirely with the need for darkness indicates that other chemical reactions must also be carried out in the dark if flowering is to take place. What these are is not yet known.

Phytochrome in short-day plants

The behavior of phytochrome provides a reasonable mechanism to explain the long-night requirement of the cocklebur. P_{FR} inhibits the chemical reactions necessary for the release of florigen from the leaves. P_R does not. Therefore the cocklebur needs 8½ hours of darkness in which (1) to convert all the P_{FR} present at sundown into P_R and (2) to carry out the unknown supplementary reactions leading to the release of florigen. If this process is interrupted by a flash of light (containing 660-nm rays), the P_R is immediately reconverted to inhibitory P_{FR} and the night's work is undone. A subsequent exposure to far-red light will convert the pigment back to P_R and steps leading to the release of florigen can be completed.

Exposure to far-red light at the start of the night sets the clock ahead two hours or so by eliminating the need for the spontaneous conversion of P_{FR} to P_R.

Phytochrome in long-day plants

In the case of short-night plants like spinach, P_{FR} is needed to stimulate the flowering process. A flash of orange-red light in the night will reverse the spontaneous conversion of P_{FR} to P_R which has been going on since sundown. This will permit flowering to occur even though the plant has been maintained under long-night conditions. In fact, spinach will bloom under continuous illumination (no night at all) when presumably all its phytochrome is in the P_{FR} form.

24.14 OTHER PHYTOCHROME ACTIVITIES

Effects of phytochrome on seed germination

Phytochrome has been found to be involved in many other plant activities. The seeds of the Grand Rapids variety of lettuce will not germinate unless exposed to light. If shortly after exposure to visible light they are exposed to far-red light, they fail to germinate. Action spectra (see Section 9.2) for this stimulation and inhibition show that phytochrome is the light-absorbing pigment. P_{FR} stimulates germination while P_R inhibits it. The same mechanism has been found to operate in the seeds of many other angiosperms, both herbaceous and woody, and in some gymnosperm seeds, e.g. those of the Scotch pine. The situation is just reversed in the seeds of the California poppy. P_{FR} inhibits germination and P_R stimulates it. Under natural conditions, these are seeds which will not germinate when exposed to light. Phytochrome is probably responsible for the light-regulated germination of the species mentioned in Section 20.9.

Etiolation

Stem growth is also regulated by phytochrome. It has long been known that plants raised in the dark elongate very rapidly. (Look back at Fig. 9.5). This phenomenon is known as etiolation. It is a mechanism that increases the probability of the plant's reaching the light. Once light shines upon it, the plant produces internodes of normal size and its leaves grow to full size. That this is not brought about by satisfaction of the plant's needs for photosynthesis is shown by the fact that exposure to light too dim to be useful in photosynthesis nonetheless halts etiolation. Orange-red light is especially effective in producing this response. This, plus the fact that exposure to far-red light causes a resumption of etiolation implicates phytochrome as the receptor in this response, too. The development of a red color in the skins of tomatoes and apples, the breaking of dormancy in some plant buds, and the straightening up of the hypocotyl arch when a dicot seedling grows above the surface of the soil (Fig. 20.11) also are triggered by light acting on phytochrome.

Phytochrome and plant aging

In the last chapter, we discussed the spontaneous aging and death that is so characteristic of annual plants once they have completed flowering and the production of seeds and fruits. In some cases, this process, too, may be a photoperiodic response involving phytochrome. Cocklebur plants that are given long nights age and die even if all their buds are removed before they can develop into flowers. If, however, the debudded plants have their

long night interrupted by light, they live several weeks longer than they normally would.

Summary

Although plants have no nervous system, they do have a variety of mechanisms by which they respond to changes in the environment. They respond to the direction of light, its wavelength, and the duration of exposure to it. They respond to gravity and temperature. All of these responses require a means (e.g. phytochrome, carotenoids) of detecting the stimulus in the environment. The detector mechanism may be located in the terminal bud or leaf or elsewhere. Once the stimulus is detected, these plants then require a communication system to enable all parts of the plant to respond in an appropriate, coordinated way. This communication system is made up of chemical messengers (e.g. auxins, florigen) transported in the contents of the phloem.

Among animals, chemical messengers also play an important role in coordinating the various organs of the body. These substances are the hormones. Their chemical nature and action is the topic of the next chapter.

EXERCISES AND PROBLEMS

1. Why do gardeners not have to worry about planting seeds upside down?
2. List six ways in which auxins affect plant function both in nature and under the control of man.
3. What term would you use to describe the closing of a crocus flower on a cold spring day?
4. What is a controlled experiment?
5. A student sprays auxin on the flowers of a tomato plant growing in a greenhouse. Tomatoes develop. Is he right to conclude that auxin promotes fruit development?
6. What is the control in the experiment illustrated in Fig. 24.17?
7. What control should have been included in carrying out the experiment shown by the righthand plant in Fig. 24.11?
8. For each of the following responses of the cocklebur, tell (1) where and how the stimulus is detected, (2) where and how the response is carried out, (3) how the information is transmitted from the first region to the second. Cite experimental evidence to support your conclusions.
   a) Phototropism of the shoot.
   b) Production of flowers when nights become at least 8½ hours long.
   c) Geotropism of the root.

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1b. "Transmission of the Phototropic Stimulus in the Coleoptile of the Oat Seedling." P. Boysen-Jensen shows that a layer of gelatin separating a coleoptile tip from its base does not interfere with the phototropic response.

1c. "On Growth-accelerating Substances in the Coleoptile of *Avena sativa*." F. W. Went proves that a growth-promoting material can be extracted from coleoptile tips.

2. Jacobs, W. P., "What Makes Leaves Fall," *Scientific American,* Offprint No. 116, November, 1955. The role of auxin in abscission.

3. Naylor, A. W., "The Control of Flowering," *Scientific American,* Offprint No. 113, May, 1952. Tells of the discovery of photoperiodism and the evidence that the stimulus is detected by the leaves and transmitted through the vascular system of the stem.

4. Salisbury, F. B., "The Flowering Process," *Scientific American,* Offprint No. 112, April, 1958. Describes experiments with the cocklebur which show that phytochrome supplies part of the clockwork by which photoperiod is measured.

5. Salisbury, F. B., and R. V. Parke, *Vascular Plants: Form and Function,* Wadsworth Publishing Co., Inc., Belmont, California, 1964. Chapter 16 describes a number of experiments that clarify the nature of photoperiodism.

6. Ray, P. M., *The Living Plant,* 2nd ed., Holt, Rinehart and Winston, Inc., 1972. Chapter 9 discusses plant hormones, and Chapter 10 describes the mechanism of photoperiodism.

25

CHEMICAL COORDINATION IN ANIMALS

Introduction

Insect Hormones

Research Techniques in Endocrinology

Human Endocrinology

The Thyroid Gland

The Parathyroid Glands

The Skin

The Stomach and Duodenum

The Islets of Langerhans

The Pituitary Gland

The Adrenal Glands

The Gonads/The Placenta

The Pineal Gland/The Thymus Gland

The Kidney

Hormones and Homeostasis

The Mechanism of Action of Hormones

The Pheromones

25.1 INTRODUCTION

Multicellular animals, like multicellular plants, must solve the problem of coordinating the activities of all the different kinds of cells of which they are made. Animals, too, need some mechanism by which the various cells, tissues and organs of the body can communicate. Only in this way can all these structures function in an efficient, well-coordinated way.

Internal communication by nerves and hormones

Two communication systems exist in most animals. One of these is the **nervous** system. It consists of specialized cells, neurons, which transmit electrical impulses from one part of the body to another. The other is the **endocrine** system. It achieves control of body functions through chemical substances, **hormones,** which are transported throughout the body in the blood. These two systems are not independent of one another. As we shall see in this chapter and the ones to follow, a close connection exists between their activities.

Chemical coordination in animals, like chemical coordination in plants, involves: (1) the release of chemicals from cells into the ECF, (2) the transport, by one means or another, of these substances, and (3) the alteration of the activities of other cells by them. Probably every cell in a multicellular organism participates in chemical coordination of this sort. In fact, we have already studied an example of this in human physiology. Every cell of our body produces carbon dioxide as a result of cellular respiration. This carbon dioxide is released into the ECF and is then carried throughout the body by the bloodstream.

When carbon-dioxide-rich blood reaches the medulla oblongata, it triggers the release of nerve impulses to the diaphragm and intercostal muscles. You remember (see Section 12.9) that the rate at which these impulses are generated governs the rate and depth of breathing. This, in turn, maintains a constant level of carbon dioxide in the blood.

There is reason to believe that all multicellular organisms carry on this kind of chemical coordination, that is, coordination achieved by release of chemical substances that are the *by-products* of other cell activities. Evidence is, however, very scanty on this point. For the present, therefore, we shall narrow our view to those special clusters of cells whose sole function seems to be the release of chemical coordinators in the body.

Endocrine glands

These clusters of cells are the endocrine glands. They are often referred to as ductless glands because their secretions, the hormones, pass directly into the blood that drains the gland (rather than into a duct as is the case with the *exocrine* glands discussed in Section 7.6). These hormones are then carried to all the other cells of the body. In some cases, they may influence the activities of all these cells. More often, the hormones exert their effect only on certain body structures, the "target" organs. So far, specific endocrine glands have been discovered only in the insects, crustaceans, certain mollusks, and all vertebrates.

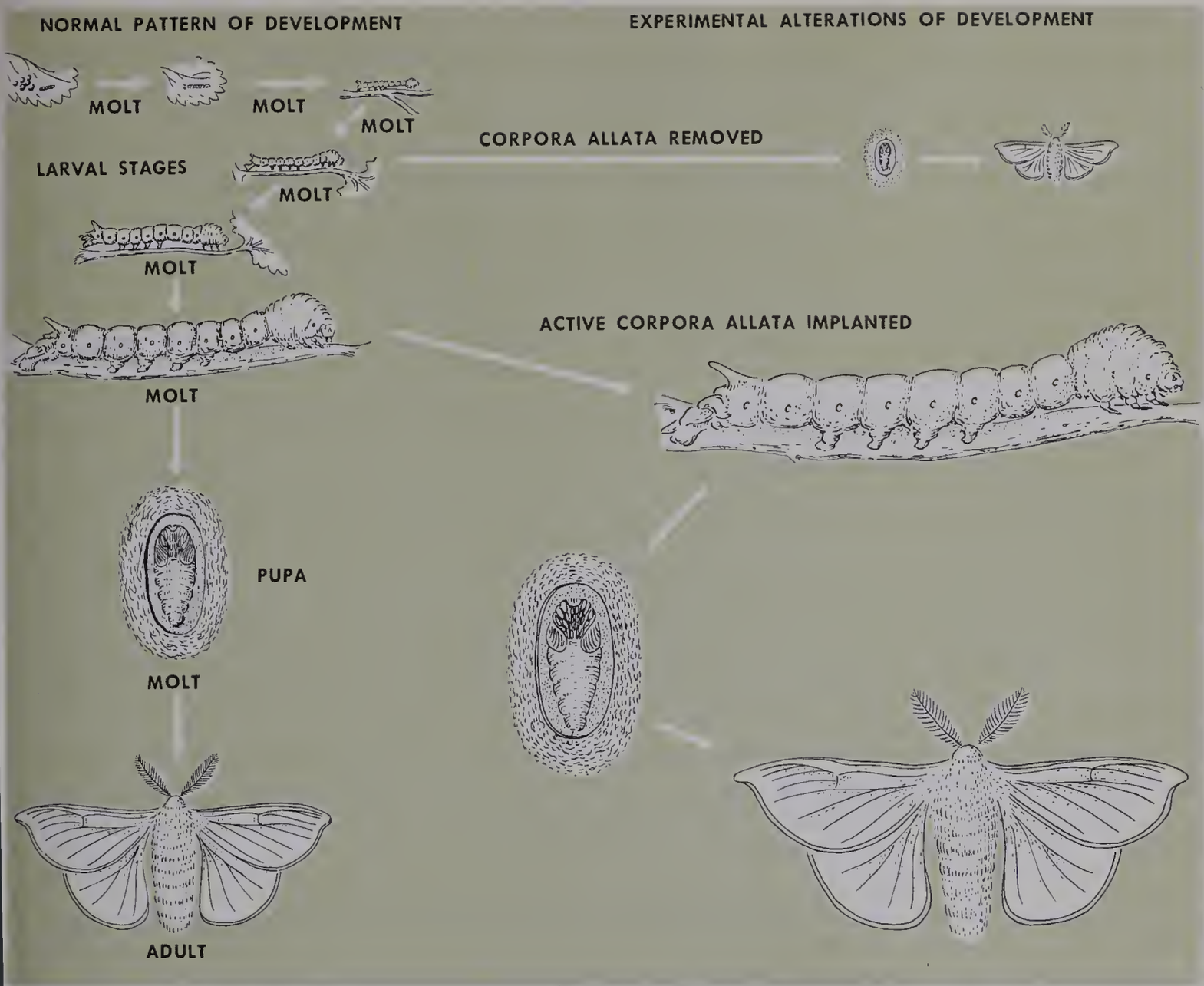


Fig. 25.1 Left: normal pattern of development in the silkworm, *Bombyx mori*. This sequence can be shortened by removing the corpora allata from a young caterpillar and lengthened by introducing active corpora allata into a mature caterpillar. Pupation does not occur so long as the corpora allata secrete substantial amounts of juvenile hormone.

25.2 INSECT HORMONES

In insects, hormones have been found which: (1) bring about color changes in the body, (2) stimulate development of eggs in the females, and (3) control growth and metamorphosis. It is interesting to note that some of these substances are produced by cells in the brain. Endocrine activity is closely related to nervous system activity in these animals.

Perhaps the control of growth and metamorphosis has been more carefully studied than any other endocrine activity in insects. Because of

Insects must molt in order to grow

their rigid exoskeleton, insects can grow only by periodically shedding the exoskeleton in the process of **molting.** This process occurs repeatedly during the period of larval development. At the final molt, the organism which emerges is the adult. In several insect orders, it bears no resemblance whatsoever to the earlier larval stages. The marked transformation in body

Metamorphosis

structure which occurs in these insects is called **metamorphosis.** It is accomplished during a dormant stage called the **pupa.** Figure 25.1 illustrates the larval, pupal, and adult stages in the development of the domestic silkworm moth, *Bombyx mori.* Metamorphosis occurs within a silken cocoon which is spun by the mature larva.

If the brain of a mature Cecropia caterpillar, one of several wild silkworms, is surgically removed before the caterpillar spins its cocoon, formation of the pupal stage does not occur. This cannot be simply a result of the shock of surgery because if the brain tissue is reintroduced into some other part of the body, pupation will proceed normally. In fact, just a tiny

The brain hormone

portion of the brain—about two dozen special cells—will do the trick. This experiment suggests that these special brain cells produce a hormone necessary for pupation to occur. This hormone does not trigger pupation directly, but instead acts upon a pair of glands in the thorax, the prothoracic glands. When stimulated by the brain cell hormone, they secrete

Ecdysone is the molting hormone

(or, possibly, cause some other tissue to secrete) a *second* hormone, a steroid called **ecdysone,** which directly initiates molting and formation of the pupa.

These two hormones, acting together, promote not only the molt from larva to pupa, but also the earlier larva-to-larva molts. What, then, accounts for the sudden change in action which occurs at the time of metamorphosis?

It has been found that if a tiny pair of glands located behind the brain, the **corpora allata** (Fig. 25.2), are removed from an immature silkworm, it spins a cocoon and undergoes pupation at its very next molt (Fig. 25.1). This raises the possibility that the corpora allata produce a *third* hormone which acts as a brake on metamorphosis. As long as substantial quantities

The juvenile hormone

of this hormone, which has been named the **juvenile hormone,** are present, ecdysone promotes larval growth. When the amount of juvenile hormone is reduced, ecdysone promotes development of the pupa. Complete absence of the juvenile hormone leads to development of the adult.

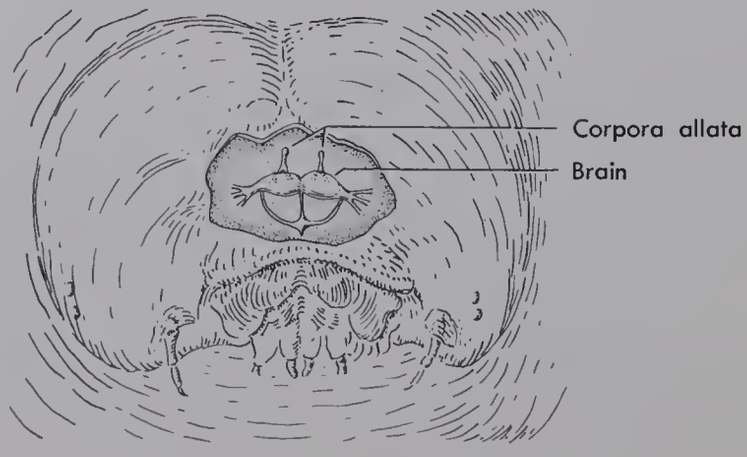


Fig. 25.2 The corpora allata of the silkworm secrete juvenile hormone. The brain itself secretes a hormone that initiates growth and metamorphosis.

This hypothesis can be tested nicely. If the corpora allata of a young silkworm are introduced into the body of a fully mature larva, metamorphosis does not occur. Molting simply results in the production of an extra-large caterpillar (Fig. 25.1). Corpora allata from a *mature* caterpillar have no such effect. Thus normal pupation seems to occur when the output of the corpora allata diminishes spontaneously in the mature caterpillar.

The development of the characteristic structures of the larva, then the pupa, and finally the adult must require the sequential participation of different sets of genes within the cells of the insect. This raises once again the question of how genes can be selectively activated or repressed. You remember (see Section 22.8, 2) that the giant chromosomes of certain flies (such as the fruit fly) have enlarged regions or "puffs" which occur at different places on the chromosomes during the course of development (Fig. 22.10). Each time a larva prepares to molt, a definite sequence of puffing occurs in its chromosomes. If ecdysone is administered to a larva shortly *after* it has completed a molt (a time when its own level of ecdysone is very low), the normal pre-molt sequence of puffing begins again. It is in these puffs that vigorous RNA synthesis occurs and, accordingly, it is the genes in the puffed regions of the chromosomes that are active. In fact, a messenger RNA has been extracted from these ecdysone-treated chromosomes that serves as the template for the synthesis of a specific enzyme not found in the cells of untreated larvae.

Evidence that ecdysone influences gene activity

The production of puffs following the injection of ecdysone is blocked by the simultaneous administration of actinomycin D. As you learned in the last chapter (see Section 24.6), actinomycin D blocks transcription of DNA, that is, the synthesis of RNA. Here, then, is additional evidence that the hormone ecdysone acts by unlocking genetic information stored within the cell.

The effect of juvenile hormone on gene expression

The response of the insect's cells to ecdysone is also influenced by juvenile hormone. As we have seen, ecdysone promotes a larva-to-larva molt when the level of juvenile hormone is high. When the level is diminished, the molt is larva-to-pupa. When juvenile hormone is absent, ecdysone promotes the development of the adult. It is as though the juvenile hormone has been repressing the genes for making adult structures. This role of juvenile hormone is beautifully illustrated by an experiment first performed by the English insect physiologist V. B. Wigglesworth. Adult insects do not normally molt, but if extra-normal amounts of brain hormone are administered to an adult "kissing bug" (*Rhodnius*), it can be forced to do so. If juvenile hormone is first applied to the insect's exoskeleton, the regions affected by it revert to larval type at this next molt (Fig. 25.3). This shows that the genes for the manufacture of larval structures are present in adult cells although normally their action is repressed.

Diapause in insects

In temperate climates, the pupal stage of many insects is formed in the fall (in response to the shorter days) and remains unchanged throughout the winter. Neither brain hormone nor ecdysone is produced during this period of **diapause.** When spring returns, so does the production of brain hormone and ecdysone. Metamorphosis is completed, and the adult

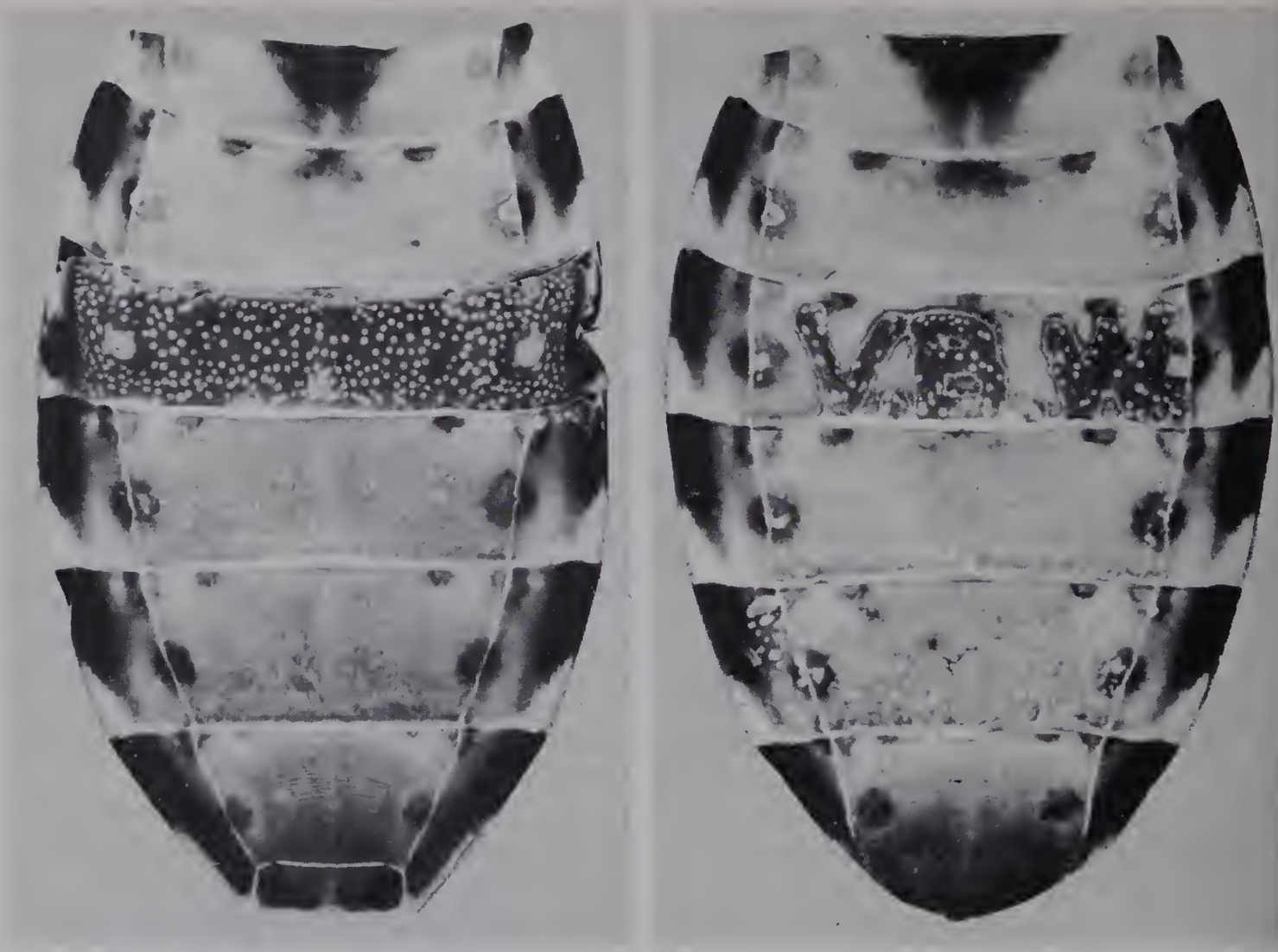


Fig. 25.3 Wigglesworth's experiment. Left: Application of a band of juvenile hormone to the cuticle of an adult *Rhodnius* results in the formation of larval cuticle when the insect is forced to undergo an extra molt. Right: The experimenter has printed his initials with juvenile hormone. (Courtesy of Dr. Wigglesworth.)

emerges from its pupal case. In at least some insects, the resumption of brain hormone secretion does not occur unless the pupa has first been exposed to a period of cold temperatures and then to warmer temperatures and increasing length of day. Cells in the brain of the pupa of one species detect the light through a transparent area of the exoskeleton just above the brain. When the days become 16 hours long, diapause is ended and development and hatching of the adult are then completed. The phenomena of enforced dormancy, a cold requirement, and photoperiodism that we examined in the last chapter are not, then, restricted to plants.

The corpora allata of insects are too small to serve as a source from which juvenile hormone can be extracted. Fortunately, many insects begin to secrete juvenile hormone once again after they are fully adult. The hormone accumulates in relatively large quantities in the abdomen of the

males of two species of silk moths. The amount accumulated is sufficiently great that it has been possible to extract the material and, after years of laborious work, to determine its chemical structure. The hormone has a relatively simple molecular structure, and we should eventually be able to synthesize substantial quantities of it or at least closely related substances.

Prospects of using juvenile hormones as insecticides

This is a particularly exciting possibility because at certain times in the life cycle of an insect, the presence of juvenile hormone leads to abnormal development and death. For example, when solutions containing juvenile hormone are sprayed on mature caterpillars, or on the foliage upon which they are feeding, normal pupation is prevented. The insect does not molt successfully into a giant larva either. Presumably this method of applying the hormone results in such an uneven distribution within the caterpillar's body that all the tissues do not respond alike. In any case, the animal soon dies. If insect eggs come in contact with even tiny traces of juvenile hormone, their normal embryonic development is upset. These phenomena have led to speculation that the juvenile hormone, if it could be synthesized cheaply and in large quantities, would make an effective insecticide. There would seem to be little chance that insects could develop resistance to a substance which is a normal constituent of their bodies. Nor does the juvenile hormone seem to have a toxic effect on other organisms (unlike such conventional insecticides as lead arsenate and DDT). On the other hand, many insects (e.g. honeybees) are beneficial to man and the use of a juvenile hormone as an insecticide might in some situations cause more problems than it cured. However, there do seem to be slight molecular differences in the juvenile hormones of different groups of insects, and different synthetic juvenile hormones show some selectivity in their action. Perhaps these differences can some day be exploited in man's unceasing warfare with the insects that destroy his crops and transmit diseases.

25.3 RESEARCH TECHNIQUES IN ENDOCRINOLOGY

The experiments which have led to our knowledge of hormonal control in insect metamorphosis provide an excellent illustration of the methods used in the study of endocrinology. The basic technique is simply this. First, an organ suspected of having an endocrine function is surgically removed from the animal's body. Second, a close observation is made of any changes or symptoms which may then occur. Third, the suspected gland is reintroduced into the animal's body to see if its presence will reverse these symptoms. If it does, the next step is to attempt to prepare an active extract (a mixture) that will duplicate the action of the missing gland. Although the extract is usually made from the gland itself, a few hormones have been extracted from such sources as urine. (The blood seldom contains enough hormone to serve as a source of supply.) Finally, an attempt is made to purify the extract and determine what single chemical substance within it produces the reversal of symptoms. This substance is the hormone.

Isolating hormones

These techniques have also been used successfully in the study of human endocrinology. Fortunately, many of the hormones found in hu-

mans are also found in the other vertebrates, so that the more drastic experimental studies can be performed on some other animal—frequently a dog or laboratory rat—with high hopes that the findings will be applicable to man. That vertebrates share many of the same hormones is also of great importance in the treatment of human endocrine disorders. Many hormones are too complex to be manufactured synthetically. They can, however, be extracted from the glands of slaughtered cattle, pigs, etc., and used in the treatment of human ailments.

The fact that vertebrates have many hormones in common does not mean that in every case they use them in the same way. As we take up the study of the hormones found in humans, we shall find some which have different functions in other vertebrates.

HUMAN ENDOCRINOLOGY

25.4 THE THYROID GLAND

The thyroid gland is a double-lobed structure located in the neck (Fig. 25.4). For its size, it has an extraordinarily rich blood supply. The most important hormone that it releases into the blood is the iodine-containing amino acid *thyroxin*. This substance has been isolated from thyroid tissue and is easily synthesized commercially. Injections of it counteract the symptoms produced when the thyroid gland is removed from experimental animals.

Functions of thyroxin

In humans, the most obvious function of the thyroid gland is the control of the rate of body metabolism. When thyroxin is administered, the amount of heat produced by the body is increased. Because energy production is a function of cellular respiration, we might expect the administration of thyroxin to increase the rate of oxygen consumption. Such is the case. In fact, measurements of oxygen consumption are used to diagnose malfunctioning of the thyroid gland.

Thyroxin in amphibians

A special function of the thyroid gland is found in amphibians. Their metamorphosis from the larval to the adult stage is triggered by thyroxin. If thyroxin (or even iodine) is administered to a small tadpole, it will undergo premature metamorphosis into a midget frog. On the other hand, surgical removal of the thyroid gland of a tadpole prevents metamorphosis.

Some races of the common tiger salamander never undergo metamorphosis from the gill-breathing larval stage to the air-breathing terrestrial form. (They reach sexual maturity and reproduce as larvae.) However, administration of thyroxin to these animals causes them to carry out metamorphosis into typical tiger salamanders. It is perhaps no accident that these so-called *axolotl* forms are found in mountain lakes in the western part of our country and in northern Mexico, where very little iodine is found in the soil and water (Fig. 25.5).

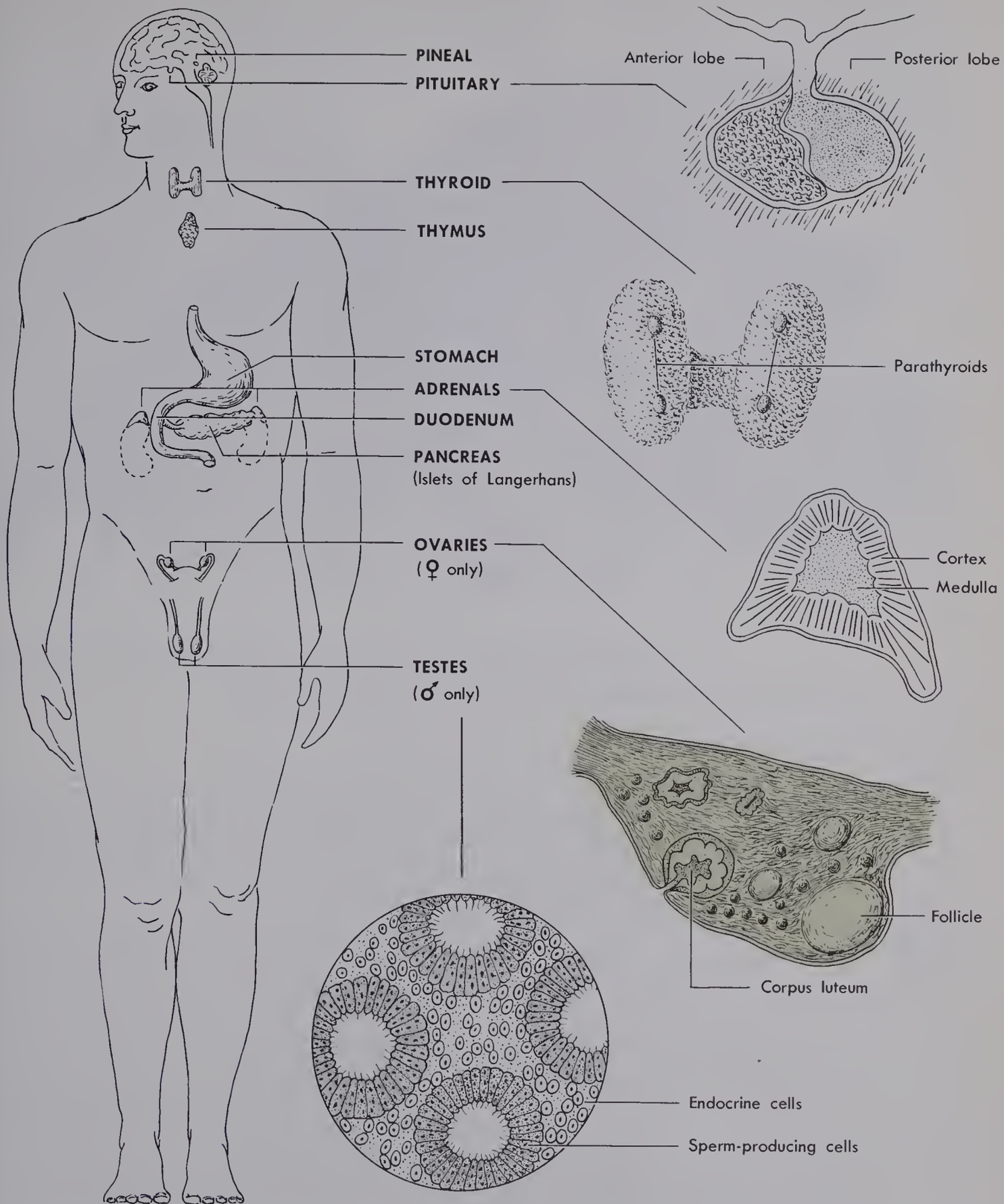


Fig. 25.4 The endocrine glands of the human. They are the same in both sexes except for the gonads, which in the female are ovaries (color) and in the male are testes.



Fig. 25.5 The axolotl, a salamander that reaches sexual maturity without undergoing metamorphosis into an air-breathing form. Note the external gills. (Courtesy of the New York Zoological Society.)

Several human diseases are associated with the improper functioning of the thyroid gland. In considering these ailments, it is important to distinguish between those associated with excessive production of the hormone (*hyper*thyroidism) and those associated with insufficient hormone production (*hypo*thyroidism).

Hypothyroidism in humans

Hypothyroidism before maturity results in the disease cretinism. The victim fails to attain either normal physical or mental development. The cause of the disease is not clear. Although it is most prevalent in areas where insufficient iodine is present in the diet, other factors are probably involved, too. In any case, all symptoms can be prevented by the early and regular administration of thyroxin. If treatment is delayed until after the symptoms have become severe, only minor improvement can be secured.

Hypothyroidism in adults causes myxedema. The symptoms of this disease are a low metabolic rate, over-weight, and a coarsening of the features. Like cretinism, it is most prevalent in iodine-deficient areas, but other causative factors are probably involved, too. It is cured by administration of thyroxin.

Iodine-deficient areas of the world (around the Great Lakes and the Pacific Northwest in the United States) are often called **goiter** belts. This

expression is used because of the large number of people with simple goiter found in these regions. A goiter is a swelling of the neck caused by a swelling of the thyroid gland. Although there is still disagreement as to the cause of simple goiter, most of the facts point to inadequate amounts of iodine in the diet. Now that iodine-rich foods (e.g. marine fish) are shipped throughout the country and iodized table salt (NaCl with traces of KI added) is used so widely, simple goiter is no longer a serious health problem.

Homeostatic control of the thyroid gland

It might seem surprising that inadequate amounts of iodine would lead to an enlargement (and hence extra activity) of the thyroid gland. It is probably a matter of compensation. The rate of activity of the thyroid gland is itself governed by another hormone, TSH, which is liberated by the anterior lobe of the pituitary gland (Fig. 25.4). An increase in the production of TSH causes an increase in the amount of thyroxin. However, an increased amount of thyroxin in the bloodstream depresses the output of TSH. This nice homeostatic device thus ensures a steady supply of thyroxin. When there is insufficient iodine in the diet for the thyroid to synthesize thyroxin, however, this control mechanism breaks down. The pituitary gland is not inhibited and thus produces ever-larger quantities of TSH. This, in turn, stimulates the thyroid gland to work harder even though it has little or no iodine to work with. Thus it becomes enlarged, and a goiter results.

25.5 THE PARATHYROID GLANDS

The parathyroid glands are four tiny structures imbedded in the rear surface of the thyroid glands (Fig. 25.4). This inconspicuous location accidentally led to the discovery of their importance. Early attempts at treating goiters by surgical removal of the thyroid gland sometimes led to unpleasant symptoms of muscle spasms and twitching in the patient. Eventually these symptoms were traced to a loss of the parathyroid glands and a resulting drop in the level of calcium ions (Ca^{++}) circulating in the blood. As we have learned, the calcium ion is one of the most important mineral constituents in the body. In addition to its role in bone formation, an adequate level of Ca^{++} in the ECF is necessary for proper functioning of the nervous and muscular systems. Too low a level of Ca^{++} in the ECF leads to the symptoms associated with removal of the parathyroids.

Metabolic actions of PTH

In 1960 a chemically pure hormone, the parathyroid hormone or PTH, was finally extracted from the parathyroid glands of cattle. It is a small protein (M.W. = 8500) containing 83 amino acid units. It produces the following effects: (1) It promotes the release of Ca^{++} from the bones. (2) It promotes the absorption of Ca^{++} from food in the intestine. (3) It promotes the reabsorption of Ca^{++} in the tubules of the kidneys. All of these actions result in an increase in the level of Ca^{++} circulating in the blood. (The hormone also inhibits the reabsorption of $PO_4^{\equiv}$ in the kidney tubules and thus helps rid the body of the excess $PO_4^{\equiv}$ produced when bone—calcium phosphate—is decomposed to provide Ca^{++}.)

Homeostatic control of the parathyroid glands

The maintenance of a constant level of Ca^{++} in the ECF (homeostasis) requires that the activity of the parathyroids be under precise control. This control is quite direct. The amount of PTH secreted by the glands is regulated by the level of Ca^{++} in the ECF. When the level of Ca^{++} drops, the glands are stimulated to release the hormone and thus restore the normal level of Ca^{++}. If the level should rise above normal, the hormone output of the gland is depressed. Here, then, is another nicely adjusted mechanism by which constancy of the internal environment is maintained.

Calcitonin

In 1961 it was discovered that the response of the parathyroids to excess Ca^{++} (the response being a subsequent reduction in Ca^{++}) is much faster than could occur simply by shutting off the production of PTH. This has led to the discovery of another hormone (named calcitonin) that acts antagonistically to PTH. This second hormone (released by the *thyroid*) provides an additional control mechanism over the Ca^{++} level in the ECF. If, for example, the Ca^{++} level of the ECF drops below normal limits, the parathyroids will be stimulated to release PTH. This is a rather slow response, however, and there is a possibility that the system might overshoot and the Ca^{++} concentration reach too high a level. Of course, depression of the glands would eventually correct this, but it might be some time before fluctuations in the Ca^{++} level were "damped out." However, release of fast-acting calcitonin prevents this overshoot and restores a steady Ca^{++} level more quickly.

Humans rarely suffer from hypoparathyroidism. Most cases in the past have resulted from accidental or unavoidable removal of the parathyroids during thyroid surgery. The unpleasant (and occasionally fatal) symptoms of parathyroid deficiency can be successfully avoided by careful addition of Ca^{++} to the patient's diet. Vitamin D, which duplicates many of the functions of the parathyroid hormone, is also used successfully in treating this disease.

Hyperparathyroidism

Occasionally one or more of the parathyroid glands becomes enlarged and extra-active. The *hyper*parathyroidism that results produces severe symptoms. The bones become brittle, weakened, and deformed. They break under the slightest stress. The great excess of Ca^{++} in the blood causes some to pass over into the urine where it may precipitate with phosphate ions and form kidney stones. These are dangerous because they occasionally block the urinary ducts. Surgical removal of the diseased glands usually brings about marked improvement.

25.6 THE SKIN

Calciferol ("vitamin D") is a hormone synthesized in the skin

When ultraviolet radiation strikes the skin, it triggers the conversion of dehydrocholesterol (a cholesterol derivative—see Section 4.2) into calciferol. Calciferol is also called vitamin D because it is present in a number of foods and such dietary sources can supplement or replace the skin as a source of supply. In its chemical structure and mode of action, however, calciferol meets all the criteria of a hormone. After synthesis in the skin, it is released to the blood, which carries it to the liver where it undergoes

one chemical modification. From the liver it travels to the kidney where it undergoes a second modification (a reaction enhanced by the presence of calcitonin). The resulting compound (1, 25-dihydroxycholecalciferol) enhances the absorption of Ca^{++} from the intestinal contents. Thus this hormone teams up with PTH and calcitonin in the regulation of calcium metabolism. Inadequate amounts of calciferol prevent the normal deposition of calcium in bone. If this occurs during childhood, deformed bones characteristic of rickets are the result. In adulthood, inadequate amounts of calciferol lead to a weakening of the bones, a condition known as osteomalacia.

25.7 THE STOMACH AND DUODENUM

There are cells in the walls of both the stomach and duodenum that display hormonal activity. As we saw in Chapter 7, the hormones produced in these cells stimulate a number of digestive processes. **Gastrin,** a polypeptide containing 17 amino acids, secreted into the blood by cells in the stomach wall, stimulates the production of hydrochloric acid by the exocrine glands of the stomach. **Secretin** and **pancreozymin** are secreted into the blood by cells in the duodenum. When these hormones reach the pancreas, they stimulate the secretion of the various components of the pancreatic digestive fluid. Secretin also stimulates the liver to secrete bile. Another duodenal hormone, **cholecystokinin** (CCK), stimulates the gall bladder to contract and force its accumulation of bile into the duodenum.

Four hormones that help regulate digestion

25.8 THE ISLETS OF LANGERHANS

The islets of Langerhans are small clusters of cells scattered throughout the pancreas in most vertebrates. There are well over a million of these clusters in the human pancreas. The cells of the islets of Langerhans are not connected to the ducts which drain pancreatic juice into the duodenum. They are, however, richly supplied with blood vessels.

As has so often been the case in science, the discovery that the islets of Langerhans are endocrine glands grew out of a chance observation. In 1889, the German physicians von Mering and Minkowski tried to learn more about the digestive functions of the pancreas by observing what digestive upsets occurred after surgically removing the organ from dogs. During the course of their studies, a laboratory assistant noticed many flies collected near the dog's urine. The urine was found to contain a large amount of the sugar glucose. The urine of normal dogs does not.

For many years, all attempts to extract a glucose-regulating hormone from the pancreas failed. However, in 1922 Dr. Frederick Banting finally succeeded in this venture. The hormone, named **insulin,** was found to be a protein. Can you see now why so many workers had failed to extract it from crude preparations of the entire pancreas? Banting succeeded where others had failed by first tying off the pancreatic ducts of his dogs. This operation caused the exocrine portion of the gland to degenerate rather

Insulin is a protein

quickly while the endocrine portion remained active. After the exocrine portion was destroyed, extracts could be made free from the digestive action of trypsin.

Insulin lowers the blood sugar level

Insulin acts to lower the level of glucose in the bloodstream. Normally, 100 ml of blood contains about 0.1 gm of glucose. After a carbohydrate-rich meal, this level tends to rise. As it does so, it triggers the release of insulin from the islets of Langerhans. The insulin passes immediately to the liver (through the hepatic portal veins), where it speeds up the conversion of glucose into glycogen and fats. As a result, the blood-sugar level is quickly brought back to normal.

One of the effects of insulin is to make the cells of the body more permeable to the entrance of glucose. Once within cells, the glucose can then be metabolized. This effect is not inhibited by actinomycin D. However, insulin also stimulates the synthesis of proteins, including enzymes that participate in carbohydrate metabolism. This action is inhibited by actinomycin D, indicating that at least one step in the inductive process is the transcription of genetic information.

Diabetes mellitus

Insufficient production of insulin results in the disease **diabetes mellitus.** Victims of this disease are unable to cope with excess glucose in the blood by converting it to glycogen or fats. Worse still, the reverse reactions are promoted. Glycogen and body fats are converted into glucose which raises the blood sugar level further. The kidney tubules fail to reclaim much of this excess glucose and so it passes out in the urine. Even body proteins are converted into glucose and then excreted. The high glucose level in the nephric filtrate exerts a powerful osmotic effect, sharply reducing the transport of water back into the blood. Consequently, victims of the disease urinate frequently and copiously. Unless corrective measures are taken, the victim wastes away, his body gradually being converted into a flood of sugary urine. Coma and death eventually follow.

Insulin therapy

Fortunately, insulin is now available in large quantities from the glands of slaughtered livestock. The molecular structure of these animal insulins is so close to that of human insulin that they may be used to treat diabetes. Unfortunately, insulin must be given by injection rather than by mouth. (Why?) Despite this inconvenience, administration of insulin to a diabetic quickly restores normal sugar metabolism in the body. Of course, insulin injections are no cure. They simply provide temporary relief from the symptoms. Nevertheless, careful attention to diet and periodic injections of insulin have enabled thousands of diabetics to lead active useful lives. In recent years at least two nationally ranked U.S. tennis players have been diabetics.

The use of insulin is not without some danger. Insulin injected after a period of exercise or long after a meal may drive the blood sugar level down to abnormally low levels. This can result in an "insulin reaction." The victim becomes irritable, fatigued, and may lose consciousness. It is quite important that those associated with diabetics learn to distinguish between the symptoms of insulin reaction and diabetic coma so that they can take proper measures if either crisis should occur.

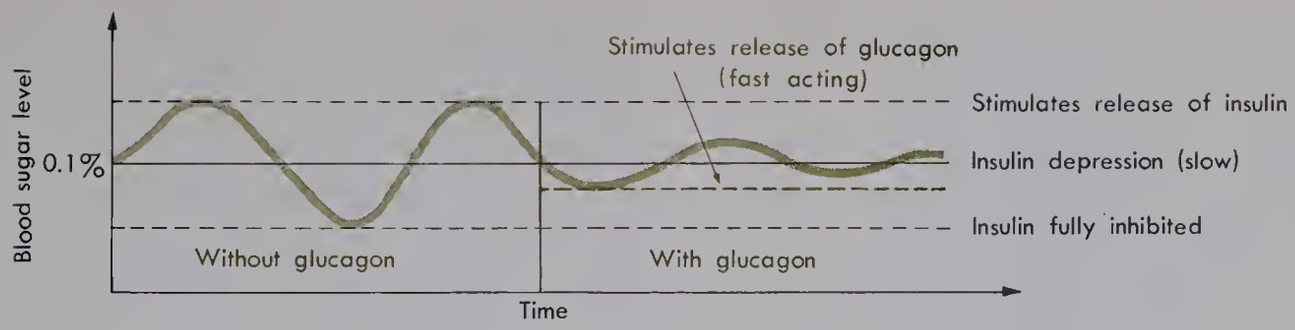


Fig. 25.6 Effect of glucagon on the homeostatic control of blood sugar. Its quick release and fast action help to prevent fluctuation of the glucose level in the blood.

Glucagon

Injection of commercial insulin preparations usually causes a brief rise in blood sugar before the longer-lasting fall. This has been found to be caused by a second pancreatic hormone, **glucagon,** which *stimulates* the conversion of liver glycogen into glucose. In the normal organism, glucagon may act to prevent insulin from lowering the blood-sugar level excessively. Its quick release and quick action dampens the tendency of the slower-responding insulin to overshoot in *its* action (Fig. 25.6). It thus plays an important role in establishing a constant level of glucose in the blood. This homeostatic role resembles that of calcitonin.

25.9 THE PITUITARY GLAND

The pituitary gland is a pea-sized structure located at the base of the brain (Fig. 25.4). In most vertebrates, it consists of three lobes: anterior, intermediate, and posterior. An intermediate lobe is present in the pituitary gland of human infants, but only a vestige remains in adulthood.

The pituitary is the master gland

Although small in size, the pituitary plays a vital role in the chemical coordination of the body. It is often called the "master" gland because many of its secretions control the activity of other endocrine glands. TSH is one example that has already been mentioned.

The Anterior Lobe

An extraordinary amount of research has been conducted on the pituitary gland. At least seven chemically distinct hormones have been isolated from the anterior lobe alone. These are:

HGH and skeletal growth

1. Human growth hormone (HGH). HGH is a polypeptide chain containing 190 amino acids. As its name implies, it promotes growth of the skeleton and the body as a whole. It does not appear to do this directly, but rather it stimulates the liver (and perhaps the kidney) to release a polypeptide that promotes the growth of muscle, cartilage, bone, and other connective tissues.

It is normally active in this respect only during the years of childhood and adolescence. A hyposecretion of HGH in the child results in stunted



Fig. 25.7 A giant and a dwarf with a man of average height. The amount of growth hormone produced during childhood and adolescence accounts for these differences.

growth or dwarfism. A hypersecretion during the same period results in gigantism (Fig. 25.7).

One cause of dwarfism may well be psychological. Orphans and other children raised under conditions where they receive little love and affection are apt not to grow normally even though their diet and other conditions of their nurturing are adequate. Although more data are needed, such children have been found to secrete abnormally small amounts of HGH. When placed in a situation where their emotional needs are met, HGH secretion and skeletal growth are restored to normal rates.

HGH is also secreted throughout adult life, especially during periods of exercise or other stress. What function it serves at these times has been the subject of intensive research. As it did during childhood, HGH continues to promote a wide variety of *anabolic* reactions within the body. In order to do this effectively, thyroxin must also be present. HGH also works synergistically with some other hormones; that is, its presence enhances their activity.

HGH has been shown to have a further function in adults: the stimulation of milk production in women after childbirth. In this it resembles the action of the next hormone to be discussed and, in humans, is very similar or even identical to it.

2. Lactogenic hormone (LTH). In mammals other than man, the lactogenic hormone is a distinctly different substance from the growth hormone. It is produced by females following childbirth and stimulates the secretion of milk from their mammary glands.

LTH has even been found in nonmammalian vertebrates. It does not, of course, stimulate milk production in these animals, but instead some kind of maternal activity appropriate to the particular species. In some birds, for example, LTH stimulates broodiness, that is, an inclination to sit on the nest. In one species of newt, it stimulates the animals to return to the water to lay and fertilize their eggs.

3. Thyroid-stimulating hormone (TSH). This hormone stimulates the thyroid gland to secrete thyroxin. The secretion of TSH is, in turn, depressed by thyroxin; thus there exists a homeostatic control mechanism over the level of thyroxin in the blood. Even the "master" gland has its controls! As we saw in Section 25.4, this mechanism can break down, producing a hypersecretion of TSH that results in goiter.

4. Adrenocorticotropic hormone (ACTH). ACTH is a polypeptide containing 39 amino acids. Its main function is to stimulate the cortex of the adrenal gland to release some of *its* hormones into the bloodstream. The vital role that these adrenal hormones play in human physiology will be considered in the next section.

In the female

5. Follicle-stimulating hormone (FSH). FSH acts upon the gonads or sex organs. In females, FSH promotes the development of follicles within the ovary (see Section 21.6). In conjunction with another pituitary hor-

mone, LH, it stimulates the secretion of **estrogens** by the follicle and the ripening of the egg within it.

In the male

FSH is produced in human males, too. In them it stimulates the development of the seminiferous tubules and the production of sperm (see Section 21.5).

6. Luteinizing hormone (LH). As a human egg matures, it completes its first meiotic division and reaches metaphase of the second division (see Section 21.1). Then it breaks out of the follicle (ovulation) and is ready for fertilization by a sperm cell. The remaining cells of the follicle become transformed into the *corpus luteum* (see Section 21.6). Every one of these activities is triggered by LH. In some mammals (including humans), LH then stimulates the corpus luteum to secrete *its* hormone, progesterone.

In the female

In the male

LH is also found in human males. It acts upon the endocrine cells in the testes (the interstitial cells—see Section 21.5) causing them to release male sex hormones (androgens) into the bloodstream.

7. Melanocyte-stimulating hormone (MSH). MSH is a small polypeptide whose sequence of amino acids shows it to be closely related to ACTH. Its "target" cells are the melanocytes, cells which contain the black pigment melanin. Large numbers of them are present in the skin, where they are responsible for moles, freckles, and suntan. Although MSH does not seem to play an important role in the normal behavior of human melanocytes, under certain conditions, such as pregnancy, an increase in its secretion does cause some darkening of the body.

In most vertebrates MSH is produced in an intermediate lobe of the pituitary gland. Its secretion causes a dramatic darkening of the skin of many fishes, amphibians, and reptiles. The darkening occurs because of the spread of granules of melanin through the branches of specialized melanocytes in the epidermis. Figure 25.8 shows these cells (called melanophores) in the skin of a frog. When the melanin is concentrated in the center of the melanophores, the skin has a light appearance. When the melanin is dispersed throughout the branches, the skin becomes much darker (Fig. 25.9). This mechanism is undoubtedly of value in enabling the animal to blend in with its surroundings.

MSH in the frog

When biologists wish to study the development of frog eggs "out of season," they initiate production of the eggs by injecting female frogs with pituitary extract. (Why?) Within minutes after the extract is injected into a frog, marked darkening of the skin occurs. This is caused by the MSH which is also present in the extract.

The Posterior Lobe

The posterior lobe of the pituitary is probably not a true endocrine gland at all. Instead of manufacturing hormones of its own, it seems simply to store those produced by nerve cells in the hypothalamus of the brain.

Two hormones have been isolated from the posterior lobe of the pituitary. **Oxytocin** is a polypeptide which stimulates contraction of

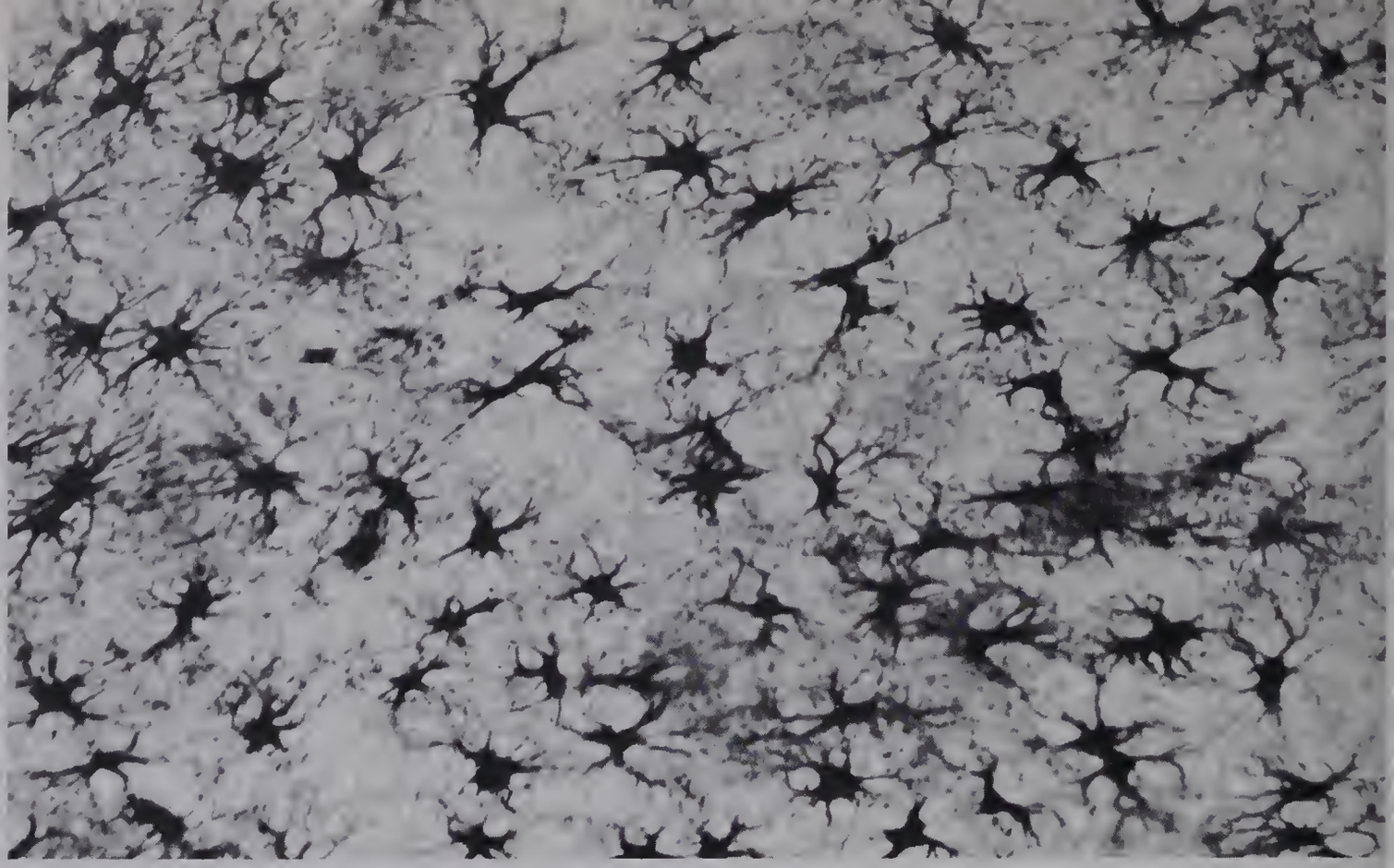


Fig. 25.8 Melanophores in the skin of a frog. The pigment melanin is dispersed throughout the branches of the cells, thus darkening the skin. The general term for any pigment-containing cell is *chromatophore.*



Fig. 25.9 MSH, acting on the melanophores, causes darkening of the frog's skin.

Oxytocin

smooth muscle, particularly the smooth muscle lining the uterus. Its secretion, probably by the baby as well as the mother, plays an important role in childbirth. Injections of this hormone are sometimes given at this time to hasten delivery of the baby and to hasten the return of the uterus to its normal size. The new mother also secretes her own oxytocin, especially if she nurses her baby.

Vasopressin

Vasopressin, a second polypeptide released by the posterior lobe, has two functions in man. It causes the muscular walls of the arterioles to contract. This constricts the bore of these vessels and causes an increase in blood pressure. Vasopressin also stimulates the reabsorption of water from the tubules of the kidneys. As we mentioned when discussing kidney function (see Section 15.6), insufficient production of vasopressin causes an enormous loss of water through the kidneys. This ailment is called diabetes insipidus. The name is derived from an early diagnostic test for the disease. The copious urine produced by hyposecretion of vasopressin is very watery and has no marked taste (insipidus). The urine produced by victims of insulin deficiency, however, contains a large amount of glucose and thus has a sweet (mellitus) taste.

Diabetes insipidus

Both the structure and the function of the pituitary gland indicate that it serves as a vital connecting link between the nervous system and the endocrine system. The pituitary is located at the base of the brain and the posterior lobe has a direct nerve connection to the hypothalamus of the brain. The anterior lobe receives blood from the hypothalamus by way of a portal system of veins analogous to the hepatic portal system (see Section 7.10). Two small polypeptides have been identified which, after synthesis in the hypothalamus, travel to the anterior lobe through these portal veins. One stimulates the anterior lobe to secrete TSH; the other stimulates the release of LH (and, probably, FSH as well), There is also considerable evidence for the existence of hypothalamic hormones that regulate the secretion of growth hormone and ACTH, although their chemical nature has not yet been firmly established. Each of these hormones seems to be synthesized in its own particular region of the hypothalamus.

Hypothalamic releasing hormones

The pituitary gland links the nervous system to the endocrine system

Pituitary secretion can often be traced to the influence of nervous stimulation. Release of vasopressin from the posterior lobe occurs when special cells in the hypothalamus detect a lowering of the water content of the blood. Release of ACTH from the anterior lobe has been shown to be partly influenced by nervous activity in the hypothalamus and is often associated with emotional states such as anger and fear. In birds, visual stimuli such as increasing length of daylight or frequent sight of a member of the opposite sex have been shown to result in an outpouring of the gonad-stimulating hormones into the bloodstream.

25.10 THE ADRENAL GLANDS

The adrenal glands are two small structures situated one atop each kidney (Fig. 25.4). They are richly supplied with blood. Both in anatomy and in

function, they consist of two distinct regions. The exterior portion is the adrenal **cortex.** The interior portion of the gland is the adrenal **medulla.**

The Adrenal Medulla

Although a true endocrine gland, the adrenal medulla is also considered to be a part of the nervous system. Its secretory cells seem to be modified nerve cells. It releases two hormones which pass into the bloodstream. The better-known of these is adrenaline. It is not yet clear what role, if any, adrenaline plays in the normal metabolism of the body. However, large quantities of this hormone are released into the bloodstream when the organism is suddenly subjected to stress such as anger, fright, or injury. As adrenaline spreads throughout the body, it promotes a wide variety of responses. The rate and strength of the heartbeat is increased, thus increasing blood pressure. A large part of the blood supply of the skin and viscera is shunted to the skeletal muscles, coronary arteries, liver, and brain. The level of blood sugar rises and the metabolic rate increases. The bronchi dilate, permitting easier passage of air to and from the lungs. The pupils of the eye dilate and there is a tendency for the body hair to stand erect. (This is particularly obvious in angered cats and dogs. Man, who is comparatively hairless, displays "gooseflesh" instead.) The clotting time of the blood is reduced, and the anterior lobe of the pituitary is stimulated to produce ACTH.

Actions of adrenaline

The second hormone of the adrenal medulla, noradrenaline, also causes an increase in blood pressure. It accomplishes this by stimulating the contraction of the arterioles.

Noradrenaline

Almost all of the body's responses to these two hormones can be seen to prepare the body for violent physical action. We have all heard accounts of heroic deeds accomplished in times of danger or other emergency. The secretion of adrenaline and noradrenaline by the adrenal medulla is an important mechanism for making such action possible.

The Adrenal Cortex

The adrenal cortex synthesizes several steroid hormones

Several different hormones have been extracted from this gland. All of them are steroids (see Section 4.2). They are quite similar in molecular structure and seem to be freely converted from one to another by enzyme action. They fall into two categories.

1. The glucocorticoids. In man, the most important members of this group are **cortisol** and the closely-related corticosterone. These hormones promote the conversion of fat and protein into intermediary metabolites that are ultimately converted into glucose. Thus they cause the level of blood sugar to rise. One of the chief target organs in this response is the liver. If cortisol is administered to an animal whose own adrenal glands have been removed, it induces in the liver the synthesis of a number of specific enzymes involved in protein and carbohydrate metabolism. This induction is blocked if actinomycin D is administered before the cortisol.

Glucocorticoids raise the blood sugar level

This effect of cortisol is, then, one that depends upon the transcription of genes.

Glucocorticoids suppress inflammation

The glucocorticoids also act to suppress inflammation in the body. Synthetic glucocorticoids have found widespread medical use in the treatment of inflammatory diseases ranging from arthritis to poison-ivy poisoning.

The glucocorticoid hormones are needed to maintain the body during periods of stress after the first brief, adrenaline-triggered response has worn off. This second, longer-lasting response seems to be quite independent of the nature of the stress. Exposure to extremes of temperature, poisoning, severe bodily injury, infection, even emotional turmoil all trigger a definite sequence of responses. The endocrinologist Hans Selye calls these responses the "general adaptation" syndrome. After the initial response to adrenaline, the body goes into a stage of "shock." The levels of glucose and salt in the blood drop sharply. Blood pressure is also reduced. Then, the adrenal cortex begins to pour glucocorticoids into the bloodstream in response to the increased secretion of ACTH by the pituitary gland. The release of ACTH is itself triggered by (1) the emotional state of the subject, (2) the lowered level of steroids in the blood, and (3) the adrenaline released by the adrenal medulla. During this phase of "countershock," the symptoms of the "shock" phase are reversed. The various body functions return to normal or even above-normal activity. The organism then enters a stage of resistance. It has become adapted to the stress.

The "general adaptation syndrome"

Mineralocorticoids maintain blood pressure

2. The mineralocorticoids. The chief function of these hormones, of which **aldosterone** is the most important in man, is to promote the reabsorption of Na^+ and Cl^- ions in the tubules of the kidneys. Not only are these ions valuable for their own sake, but their retention in the blood keeps its osmotic pressure high. This, in turn, assures normal blood volume and pressure.

Aldosterone secretion is regulated by angiotensin II

The rate of aldosterone secretion is controlled by a number of factors. The most important of these is the level of angiotensin II in the blood. In Chapter 13, we examined the mechanism by which a drop in blood pressure causes the kidney to secrete renin, which, in turn, leads to the production of angiotensin II (see Section 13.20). The stimulatory effect of angiotensin II on aldosterone secretion thus provides a negative feedback device by which the concentration of Na^+ in the ECF is maintained. The rate of aldosterone secretion is also enhanced somewhat by the presence of ACTH and a high level of K^+ in the ECF.

Mineralocorticoid hormones are found in other vertebrates, too. In every case, they also function in the control of salt and water balance in the animal. The exact action varies, however, according to the environment of the organism. In the freshwater fishes, they act to retain salts but release water. This action is just reversed in the marine fishes, with the hormones promoting the excretion of salt by the gills and the reabsorption of water by the kidneys. The active excretion of salt by the nasal glands of marine birds and reptiles is also stimulated by them. (See Section 6.4.)

Mineralocorticoids in fish

Evidence that aldosterone activates genes

The active transport of Na^+ ions has been intensively studied in the bladder cells of the toad. It has been demonstrated that the phenomenon depends upon the induction, by aldosterone, of enzyme synthesis in the cytoplasm of these cells. However, when aldosterone is administered to the cells, it moves into the nucleus. Only after about an hour is the transport of Na^+ ions increased. The response to aldosterone is inhibited by actinomycin D, and this supports the idea that an early effect of the hormone is on the genetic code.

The glucocorticoids and mineralocorticoids are essential to life. Under laboratory conditions, some animals can survive for about two weeks when their adrenal glands have been surgically removed, but they must be protected from stress. The slightest stress of any kind causes a sudden drop in the sugar and salt levels of the blood, and death follows quickly. Humans cannot survive even under the best conditions without their adrenal cortices. If absolutely no cortical steroids are present, death comes in a few days. A hyposecretion of these hormones results in Addison's disease. The symptoms are lowered blood pressure, loss of appetite, muscular weakness, and general apathy. Unless corrective measures are taken, death follows after about two years. Fortunately, administration of synthetic cortical steroids and/or ACTH can today restore the patient to a relatively normal life.

Addison's disease

25.11 THE GONADS

Both the male and female gonads (Fig. 25.4) possess endocrine activity in addition to their prime function of producing the sex cells.

The Testes

The testes of males contain endocrine tissue, the interstitial cells. When stimulated by the anterior pituitary hormone LH, these cells release androgens (e.g. **testosterone**) into the bloodstream. This action commences at the start of adolescence. Testosterone triggers the development of the so-called secondary sexual characteristics found in adult males. The voice deepens. Hair begins to grow on the face, in the armpits, and around the genital organs. These organs become enlarged. The body takes on the general angular contours typical of adult males. The formation of fat deposits is inhibited.

Androgens stimulate the development of the secondary sex characteristics of males

Castration

Removal of the testes (castration) before this period prevents the development of the secondary sexual characteristics. Until close to the end of the nineteenth century, this operation was occasionally performed on Italian boys. The object was to preserve their soprano voices for church choirs. Castration has also been practiced in certain Near Eastern countries to provide eunuchs to guard the royal harem. The response of adult males to castration varies, but generally there is only a moderate loss of masculine traits. Castration is frequently used in animal husbandry to provide animals with better meat quality and/or easier temperament. Castrated cattle, horses, and fowl are called steers, geldings, and capons respectively.

Anabolic steroids

A number of synthetic androgens are marketed for certain therapeutic uses. Some evidence has been presented that among the effects of these drugs is an increase in body weight and muscular strength. Despite the fact that many investigators do not agree that these effects occur, use of these drugs is now frequently found among certain athletes, especially weight-lifters, shot-putters, and professional football players. There is a genuine question not only as to whether the drugs do have the anabolic effects that some ascribe to them, but also as to their safety.

The Ovaries

The ripening **follicle** in the ovary not only contains a ripening egg but also acts as an endocrine gland. The accessory cells of the follicle liberate several steroid hormones called **estrogens.** They are stimulated to do so by the combined influence of FSH and LH from the anterior lobe of the pituitary gland.

Estrogens and the secondary sex characteristics of females

The estrogens have two major functions in the female body. First, they promote the development of the secondary female sexual characteristics. At the start of adolescence, the mammary glands (breasts) develop, the pelvis becomes broader, the genital organs enlarge, and hair develops around them and in the armpits. The estrogens are responsible for these changes. They also promote the development of fat deposits and these lead to the more rounded body contours characteristic of adult women.

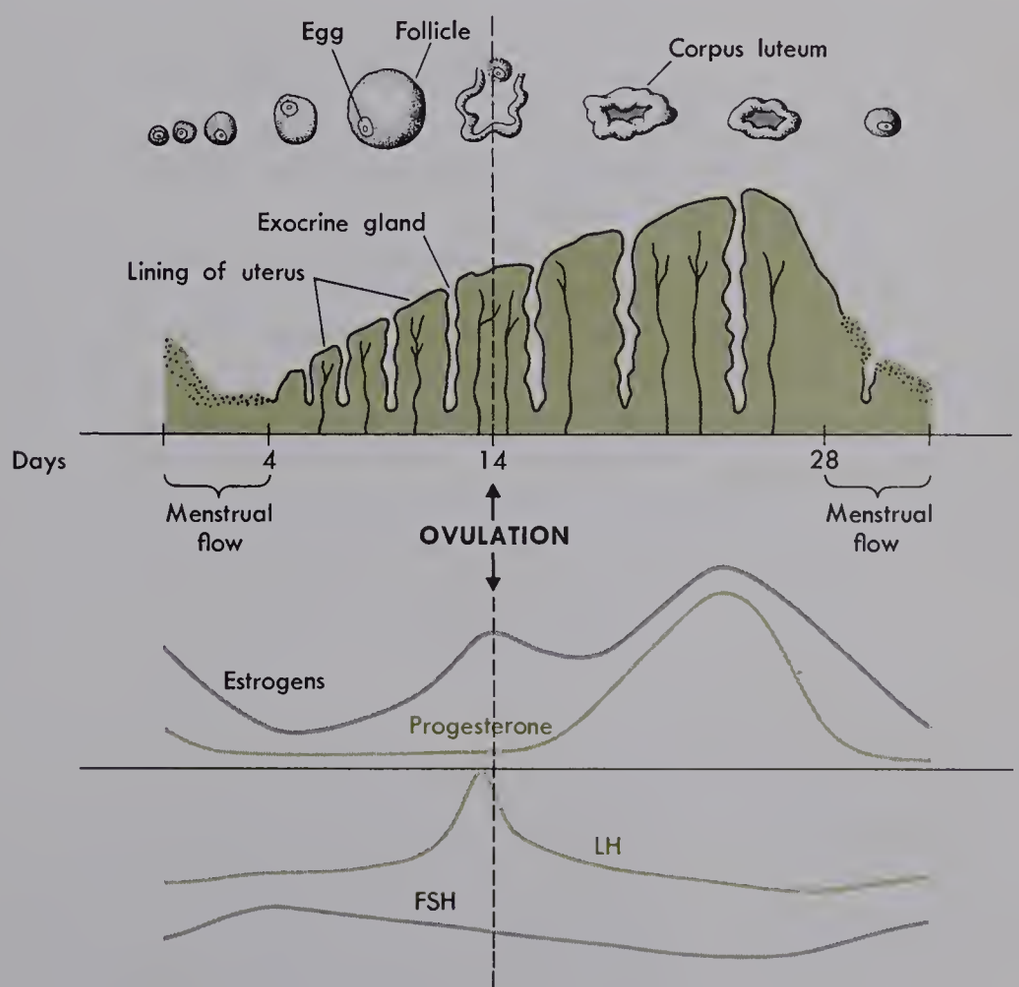


Fig. 25.10 Events occurring in the ovary (top), changes in the lining of the uterus (color), and changes in hormone levels in the blood during the menstrual cycle.

Estrogens and the menstrual cycle

The estrogens continue to be secreted throughout the reproductive years of women. During this period they participate in the monthly preparations of the body for possible pregnancy. This includes the preparation of the inner lining of the uterus to receive an embryo. The lining becomes thicker (Fig. 25.10) and more richly supplied with blood. In response to estrogens, its cells become very active, first in RNA synthesis, and then in protein synthesis. These responses are blocked by actinomycin D, so they depend on the transcription of genes.

The corpus luteum secretes progesterone

The corpus luteum (which is produced after ovulation—see Section 21.6) is also an endocrine gland. Stimulated by LH, it secretes **progesterone** into the bloodstream. This hormone continues the preparation of the uterus for pregnancy and inhibits the development of a new follicle.

FSH and LH

The secretion of estrogens is stimulated by FSH and LH, while the secretion of progesterone is stimulated by LH alone. Both estrogens and progesterone act back on the hypothalamus, inhibiting its secretion of the releasing factor(s) for FSH and LH. Thus production of FSH and LH by the anterior pituitary is inhibited. These homeostatic interactions (similar to that between TSH and thyroxin) serve to coordinate estrogen and progesterone production to the needs of the monthly sex cycle. They also provide

The "pill"

the physiological basis for the effectiveness of "the pill" as a contraceptive agent. The pill contains both a synthetic estrogen and a synthetic progesterone. Like the natural hormones, these substances act on the hypothalamus, inhibiting the secretion of releasing factor(s) and hence of FSH and LH production by the pituitary. Without FSH and LH, neither normal follicle development nor ovulation can occur. The pill also inhibits the steps which would otherwise enable an egg to move down the Fallopian tube and, if fertilization had occurred, become implanted in the lining of the uterus.

The pill is generally taken for about three weeks (sometimes estrogens alone are used during the first two weeks of this period). Then, stopping the pill for a week allows normal menstruation to occur.

The menopause

At the end of a woman's reproductive years (the menopause), the production of eggs and estrogens ceases. Disappearance of the estrogens removes their brake on the pituitary, and large quantities of FSH are liberated into the bloodstream. The control mechanism has broken down. The elevated level of FSH causes a variety of unpleasant physical and emotional symptoms. Many doctors now administer estrogens at this time, in order to reimpose a brake on FSH secretion by the pituitary and thus relieve the symptoms.

25.12 THE PLACENTA

Nourishment of the human embryo while within its mother's uterus is accomplished through the umbilical cord and placenta which connect the embryo indirectly to the mother's circulatory system (Fig. 21.12). After pregnancy is established, the placenta takes on the secondary function of serving as an endocrine gland. It secretes estrogens, progesterone, and

another hormone quite similar to the gonad-stimulating hormones of the anterior lobe of the pituitary. These hormones supplement those produced by the corpus luteum and the pituitary gland. As the time of birth approaches, the placenta also secretes high levels of **relaxin,** which, as we learned in Section 21.7, makes a more flexible passageway for the exit of the baby.

25.13 THE PINEAL GLAND

Melatonin

The pineal gland is a small, pea-sized structure attached to the brain just above the cerebellum (Fig. 25.4). It produces a hormone called **melatonin.** When this hormone is injected into frogs, it causes a marked lightening of the skin. Its action on the melanophores (Fig. 25.8) is thus just the reverse of that of MSH. In the laboratory rat and the hamster, melatonin inhibits the gonads both as gamete producers and as endocrine organs. The secretion of melatonin in these animals is markedly increased when they are placed in the dark and decreased when they are exposed to light. Many mammals have inactive gonads during the winter. With the lengthening days of spring, their gonads become active once again and mating follows. Perhaps the pineal gland, through its secretion of melatonin, serves as a connecting link between the eyes and the gonads in this **photoperiodic** response. As for humans, its normal role is still uncertain.

25.14 THE THYMUS GLAND

The thymus gland consists of two lobes of tissue similar to that found in lymph nodes. It is located high in the chest cavity just under the breastbone (Fig. 25.4). It is large during childhood but then shrinks after the start of adolescence. Many techniques, including surgical removal, have been employed in attempts to find an endocrine function of the thymus, but none has been convincingly demonstrated in adult mammals. However, there is good evidence that the thymus in the infant mammal plays a major role in setting up the lymphocyte-producing machinery of the lymph nodes, thus providing the basis for the development of antibodies. This seems to involve, among other things, the release of a hormone (as yet unidentified) into the bloodstream. Once the job is completed, the thymus is ordinarily no longer needed.

The thymus may secrete a hormone necessary for the establishment of immunity

25.15 THE KIDNEY

The kidney secretes at least three hormones

The kidney has at least three endocrine functions. In Sections 13.20 and 25.10, we examined the role that its secretion of renin plays in maintaining blood pressure. In Section 25.6, we noted that the kidney completes the conversion of calciferol into its most effective form. The kidney also secretes **erythropoietin** into the bloodstream, especially in response to anemia. Erythropoietin acts on the bone marrow to increase its production of red blood cells (RBC's).

25.16 HORMONES AND HOMEOSTASIS

Our study of the human endocrine system reveals what a variety of important roles are accomplished by chemical coordination. Most (but not all) of these chemical controls act in a rather slow, generalized way. Growth, sexual development, and metabolism are three body processes under hormonal controls that act gradually over a span of time.

The endocrine system also plays a major role in the maintenance of a constant internal environment. The concentration of sugar, water, and various salt ions in the ECF is maintained within narrow limits by hormonal action. There are at least three controlling mechanisms by which hormones maintain homeostasis in the body.

Hormonal control mechanisms

1. The secretion of some hormones is directly controlled by the need for that hormone. A high level of Ca^{++} in the blood suppresses the output of PTH. A low level of Ca^{++} stimulates it. The level of sugar in the blood acts directly on the islets of Langerhans, promoting the appropriate response from them. The osmotic pressure of the blood triggers (with the aid of the nervous system) the production of vasopressin and thus its own readjustment.

2. In some cases, the response of a gland to the level of the substance it regulates is apt to be slow. The resulting lag in response may cause undesirable fluctuations above and below the desired level. This situation may be improved by a second hormone acting antagonistically to the first. The antagonistic action of such pairs of hormones as insulin-glucagon and PTH-calcitonin provides a system of checks and balances for reestablishing homeostatic equilibrium quickly after any displacement.

3. A third system for achieving self-regulation of hormone production is illustrated by the relationship between TSH and thyroxin. Whenever one hormone stimulates the production of a second, we find that the second hormone acts, in turn, to suppress the production of the first. Another example of this is the way in which high levels of estrogens keep the production of FSH in check. Here again is a self-contained control system (analogous to the action of a governor on an engine) for maintaining homeostasis.

25.17 THE MECHANISM OF ACTION OF HORMONES

All the human hormones we have studied in this chapter are transported by the bloodstream. They are thus carried from the endocrine gland which produces them to every cell of the body. In some cases (e.g. thyroxin), every cell of the body responds to the presence of the hormone. More often, only certain cells, those in the "target organ," respond. This arrangement can be compared with that in radio broadcasting. We live surrounded by the electromagnetic radiation from a large number of broadcasting transmitters. However, only a correctly tuned receiver can respond to this energy. In a similar way, only certain of our cells may be competent to respond to a given hormone circulating in the blood.

Target organs

The matter of cell competence cannot be overly stressed. Hormones, in themselves, cannot accomplish the various functions we have ascribed to them. Hormones simply release within the target cells their potentialities for dealing with the situation. Insulin added to blood in a test tube has no effect on the glucose content. In the living organism, however, it enables the cells to reduce the blood-sugar level.

Hormones and gene action

Throughout the chapter we have noted that many hormones include among their effects a stimulation of protein synthesis. Usually this effect is inhibited by actinomycin D. In view of the role that actinomycin D plays in preventing DNA transcription (i.e. RNA synthesis), we conclude that these hormonal effects involve the derepressing of gene action. While it is tempting to think that hormones might achieve this effect by combining with a specific repressor molecule just as lactose does with the repressor of the lactose operon in *E. coli* (see Section 22.8), there is little evidence that such is the case.

In trying to discover the mechanisms of hormone action, it has become apparent that our hormones fall into two major categories. The steroid hormones (e.g. estrogens, cortisol, aldosterone, calciferol) *enter* their target cells. In several cases, it has been shown that, once within the cell, the hormone quickly moves into the nucleus (probably bound to a cytoplasmic protein). Once within the nucleus, the hormone-protein complex binds to DNA (at least in the cases of estrogen and cortisol). It does not appear to be acting as a repressor, however, because these events induce, not repress, gene activity. Trying to find out just what role these complexes play in the inductive process is an extremely active field of research.

Steroid hormones enter their target cells

The protein and polypeptide hormones (and also amino acid-derived hormones like adrenaline) make up the second major category. These hormones (which include insulin, MSH, PTH, ACTH, glucagon, TSH, vasopressin, and LH) may also induce gene activity, but the mechanism at work is quite different. They do not enter their target cells but, instead, bind to specific receptors on the cell surface. In so doing, they activate an enzyme attached to the membrane called adenyl cyclase (Fig. 25.11). Adenyl cyclase catalyzes the conversion of ATP in the cytoplasm into cyclic AMP (Fig. 25.12). An extraordinary variety of cell functions are affected (either stimulated or inhibited) by a rising level of cyclic AMP within the cell. The particular functions affected in any given cell depend on the type of cell involved, that is, on its past history of differentiation. Thus cyclic AMP stimulates the β-cells of the Islets of Langerhans to release insulin, kidney cells to secrete renin, liver cells to convert glycogen into glucose, thyroid cells to release thyroxin, and so on. Cyclic AMP and its role as a "second messenger" in hormone action were discovered by Earl Sutherland. In 1971 he was awarded the Nobel Prize for these discoveries.

Cyclic AMP

While many (not all) of the actions of hormones can be traced to the activation of genes, the mechanisms at work are surely more complex than those discovered in *E. coli*. Even if some hormones do interact quite directly with DNA, the fact that they can do so only in certain "target" cells (e.g. estrogen in the cells of the uterus) and not in others tells us that

In many cases, the capacity of a cell to respond to a hormone is acquired during differentiation

something more is involved. We have established that every cell in the organism has a complete library of genetic information. Why, then, can certain genes be expressed in some cells but not in others? The answer is unknown, but surely it is an outcome of the history of the cell, that is, the process of cellular differentiation itself. When we finally understand the gene control mechanisms by which a cell becomes differentiated, we shall be much closer to an understanding of the diverse effects that hormones have on them.

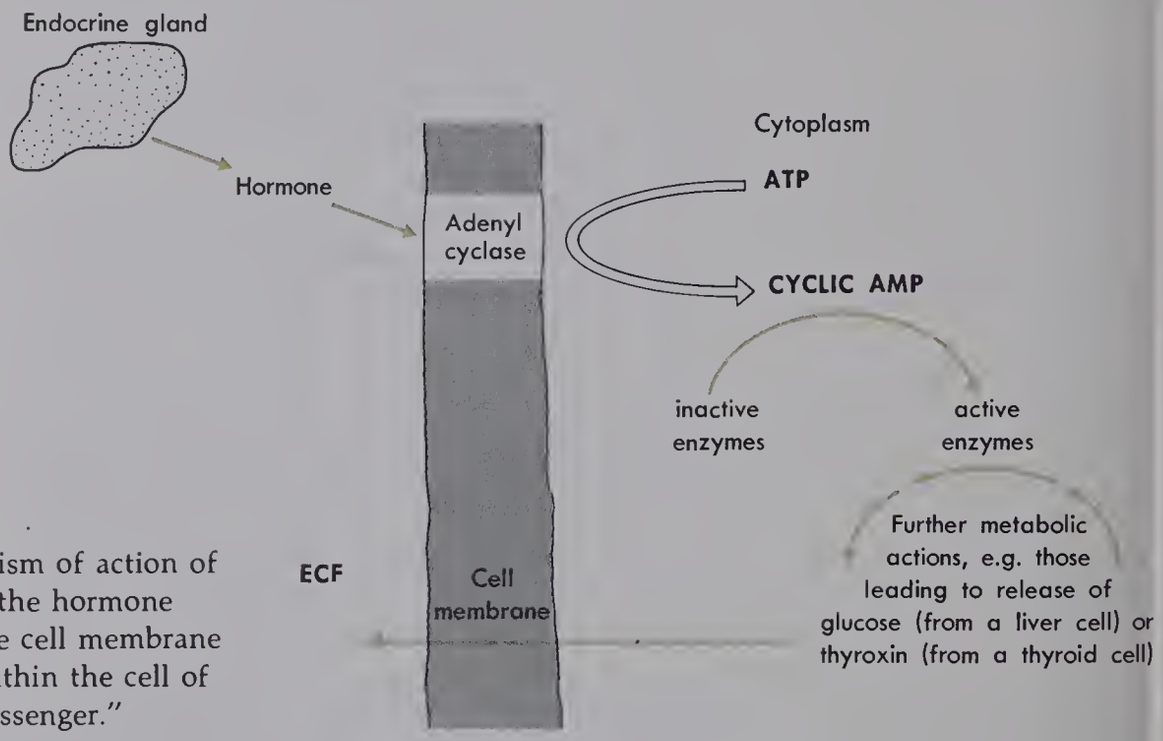


Fig. 25.11 Probable mechanism of action of some hormones. Binding of the hormone (the "first messenger") to the cell membrane stimulates the production within the cell of cyclic AMP, the "second messenger."

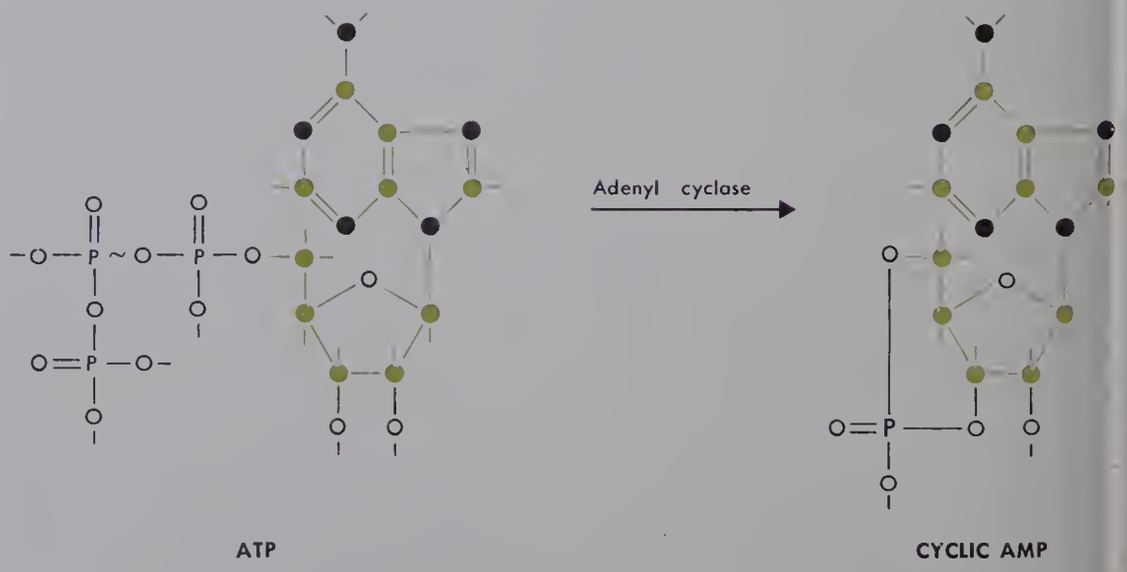


Fig. 25.12 Conversion of ATP to cyclic AMP. (Carbon atoms are shown in color, nitrogen atoms in black, and hydrogen atoms as short dashes.)

25.18 THE PHEROMONES

Hormones are chemical substances that are released into the *internal* environment (ECF) by *endocrine* glands. Carried throughout the body, they coordinate many of the activities of its various parts. This coordination provides, among other things, for a close regulation of the chemical properties of the internal environment and the activities of our internal organs.

Pheromones are chemical signals with which different members of a species can communicate

In recent years, an entirely different category of chemical coordinators has also been receiving careful study. These are the **pheromones.** Pheromones are chemical substances which are released into the *external* environment by *exocrine* glands. They provide a means of communication with other members of the same species.

Some pheromones produce gradual effects

In some cases, the communication is quite subtle. The pheromones released by some members of the species simply initiate physiological changes in the others. These changes usually do not lead to any externally directed response for some time. Adult male grasshoppers of the species that migrates in locust plagues release a pheromone which, when detected by immature members of the species, hastens their growth. This, in turn, speeds the time when a migratory swarm will be formed and a locust plague will get under way. The queen bee in a honeybee colony secretes a substance which prevents the worker bees from developing ovaries and laying eggs. However, if the queen is killed or removed from the hive, the disappearance of this pheromone permits some of the workers to take over her egg-laying function.



Fig. 25.13 A stick treated with the trail pheromone of an ant (left) can be used to make an artificial trail which is followed closely by other ants emerging from their nest (right). The trail will not be maintained by the other ants unless food is placed at its end. (Courtesy of Sol Mednick and *Scientific American.*)

Some pheromones produce immediate effects

Other pheromones have been discovered which trigger immediate action when detected. When an ant is disturbed, it secretes from glands in its head a volatile chemical that quickly diffuses in all directions. This chemical can be detected by other ants several centimeters from the spot. They are attracted by low concentrations of the substance and begin to move toward the area of increasing concentration. As they thus get nearer their disturbed nest mate, their response changes to one of alarm. The higher concentration of the pheromone causes them to run about actively as they remedy the disturbance. Unless additional quantities of this pheromone are released, it soon dissipates. This is important, too, so that once the emergency is over, the ants can return quietly to their former occupations.

Several other ant pheromones have been discovered, including one that is laid in a trail by a worker returning to the nest with food. This trail attracts and guides other ants to the food source (Fig. 25.13). It is continually renewed as long as the food holds out. When the supply begins to dwindle, however, trail-making ceases. The trail pheromone evaporates quickly so other ants stop coming to the site and will not be confused by old trails when food is located elsewhere.

Social insects have an especially elaborate repertoire of pheromones

Most of the pheromones discovered so far have been in insect species, although a few have been found in other animals. Furthermore, the most elaborate pheromone communication systems have been found in the social insects—the ants, termites, and bees. This should not be surprising when you remember the argument we examined in Section 5.24, that societies represent one *further* stage of biological organization beyond the levels of tissue $\longrightarrow$ organ $\longrightarrow$ system $\longrightarrow$ organism. Just as hormones help to coordinate the action of the tissues, organs, and systems within the organism, so pheromones help to coordinate the action of the individuals that make up a society.

A response to a pheromone is a response to one of many kinds of stimuli present in an animal's external environment. In order to detect the stimulus and to carry out an immediate, coordinated response, a nervous system must be present. We now turn our attention to the general topic of nervous coordination.

EXERCISES AND PROBLEMS

1. Summarize the mechanisms which participate in the control of the blood-sugar level in the body.
2. Glucocorticoid therapy for children is undesirable because it may result in severe stunting of growth. What mechanism might be responsible for this?
3. Why must insulin be given by injection rather than by mouth?
4. How will each of the following affect the quantity and composition of urine formed in humans: (a) hypersecretion of vasopressin, (b) hyposecretion of insulin, (c) drinking copious amounts of water?

5. Which endocrine glands are controlled by the secretion of other endocrine glands?
6. What parallels can you find between the relationship of organs in an organism and the relationship of individuals in a society?
7. How is communication among the parts of an organism accomplished?
8. How is communication among the parts of a society accomplished?
9. What is the significance of diapause in insects?
10. Compare the mechanisms by which (a) the body regulates the concentration of water in the blood and (b) a steady temperature is maintained in your home.

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26 NERVOUS COORDINATION: STIMULUS RECEPTORS

The Three Components of Nervous Coordination

The Compound Eye

Structure of the Human Eye

Detection of Light

Heat Receptors

Touch and Pressure

Hearing

Equilibrium

Taste

Smell

Internal Chemical Receptors

Summary

Nervous coordination differs from endocrine coordination in being faster and generally more localized in its action. It enables the organism to respond quickly to changes in the external as well as the internal environment. This is in contrast to the endocrine system which, as we know, is primarily concerned with internal changes. Although both plants and animals carry on chemical coordination by means of transported hormones, nervous coordination is characteristic of animals only.

26.1 THE THREE COMPONENTS OF NERVOUS COORDINATION

Receptors detect stimuli

The ability of an organism to respond to changes in its environment requires the presence of three different components. *First,* there must be a stimulus **receptor.** This is a structure capable of detecting a certain kind of *change* in the environment and initiating a signal, the nerve impulse, in the nerve cell to which it is attached. Our sense organs are stimulus receptors. They enable us to detect such stimuli as light (the eye), sound (the ear), and chemicals (the odor receptors in the nose).

Nerves conduct impulses

The *second* component in nervous responsiveness and coordination consists of the impulse **conductors,** the nerves themselves. Nerves are made up of bundles of conducting fibers in much the same way that a telephone cable is made up of bundles of wires. These fibers are greatly elongated extensions of special cells, the **neurons.** Two kinds of neurons make up most nerves. **Sensory** neurons transmit impulses from the stimulus receptor to the central nervous system, the brain and spinal cord. **Motor** neurons transmit impulses from the central nervous system to the part of the body that will take action.

In a few cases, sensory neurons may transmit their impulses directly to motor neurons, the junction occurring in the central nervous system. Most of the time, though, impulses from sensory neurons pass along one or many **association** neurons before finally reaching a motor neuron. The central nervous system is composed of millions of these interconnecting association neurons. Their complex arrangement provides a virtually unlimited number of routes by which impulses can travel through the central nervous system. This, in turn, enables a great variety of complex actions to be carried out in a well-coordinated way. If the nerves can be compared to telephone cables, the central nervous system should be compared to the switchboards of the telephone exchange.

Muscles and glands take action

The *third* component in nervous coordination consists of the **effectors.** These are the structures that carry out some action in response to impulses reaching them by way of motor neurons. The most important effectors in man are the muscles and the glands (both exocrine and endocrine).

In this chapter we shall examine the various kinds of stimulus receptors. Some of these detect stimuli arising within the body. Often, but not always, the nerve impulses generated by them are not made conscious in the brain. Other receptors detect stimuli in the external environment. Generally, these stimuli give rise to conscious sensations. It is important to realize that sensations exist only in the brain and not in the organ that

The brain is the seat of all sensation

detects the stimulus. Under general anesthesia, there are no sensations. Stimuli are still detected and nerve impulses are still sent back to the central nervous system. But the brain is unable to assign any interpretation to these impulses and hence no sensation exists. On the other hand, a person whose leg has been amputated may still feel pain ("phantom" pain) in the missing leg. In such a case, remnants of sensory neurons in the stump continue to send impulses back to the brain. It is the brain alone which interprets these impulses as signifying pain in the now-missing structure.

Most animals have receptors of radiant energy **(photoreceptors),** mechanical forces **(mechanoreceptors),** and chemicals **(chemoreceptors).**

PHOTORECEPTORS

Evolution has produced receptors for only a limited range of wavelengths of electromagnetic radiation

Radiant energy exists in a range of wavelengths that extends from radio waves that may be thousands of yards in length to gamma rays with wavelengths as short as a million millionth (10^{-12}) of a meter (Fig. 26.1) Over this long span, the only wavelengths that generally serve as stimuli for living things are light rays (about 400–700 nm) and the somewhat longer infrared or heat rays.

Light receptors are common in the animal kingdom. They range in complexity from light-sensitive cells which simply detect the presence of light (as in the earthworm) to eyes that are capable of forming images. The latter are found in certain mollusks (especially the squids and octopuses), most arthropods (insects, crustaceans, and arachnids) and the vertebrates.

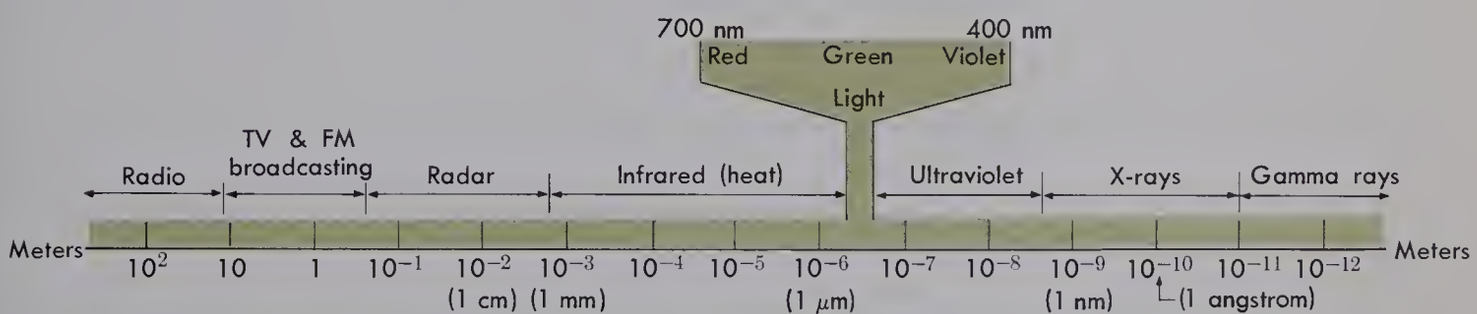


Fig. 26.1 The spectrum of electromagnetic radiation.

26.2 THE COMPOUND EYE

The ommatidium

The structure and functioning of the arthropod eye is quite different from that of the mollusk and vertebrate eyes. It is called a compound eye because it is made up of repeating units, the **ommatidia,** each of which functions as a separate visual receptor. Figure 26.2 shows the arrangement of ommatidia in a compound eye. Each ommatidium consists of (1) a lens (the front surface of which makes up a single facet of the compound eye),

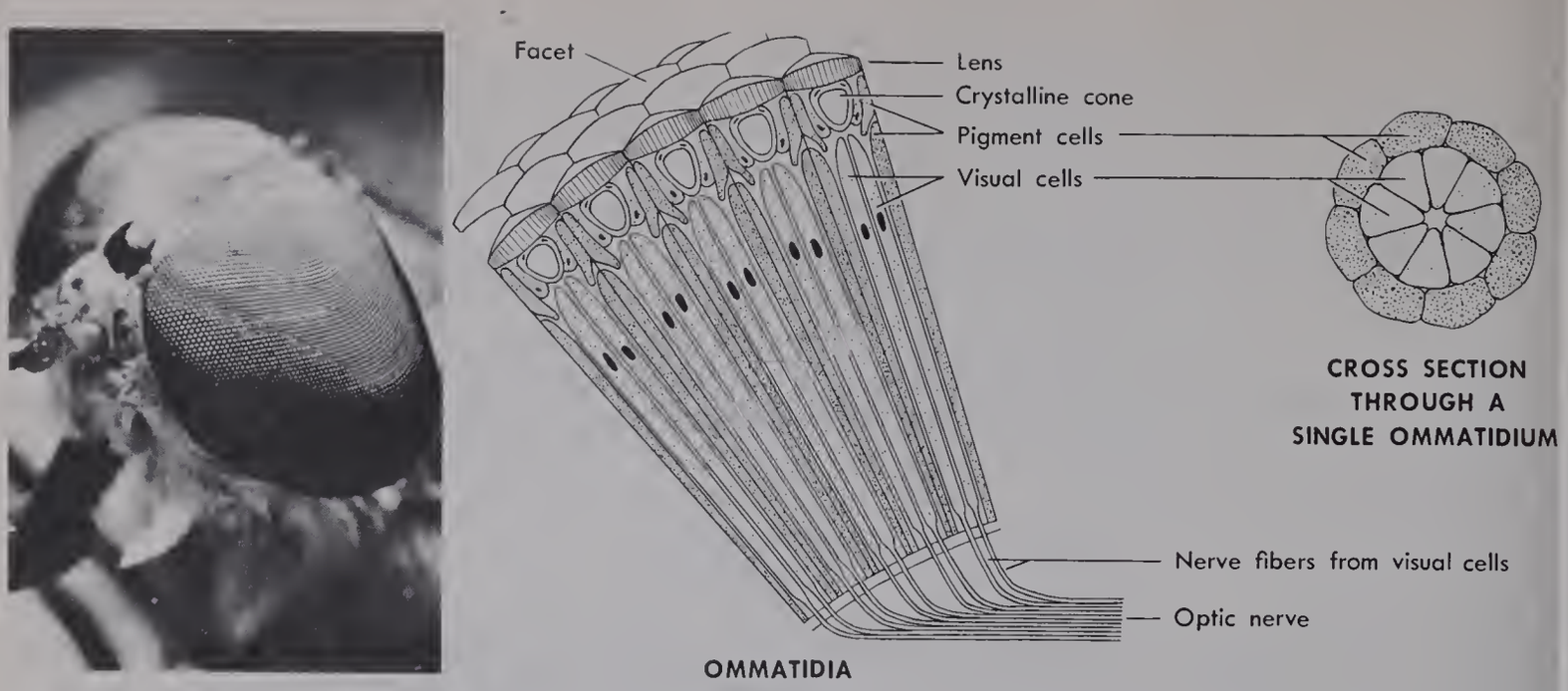


Fig. 26.2 Left: compound eyes of a tabanid fly. They are made up of thousands of ommatidia (center), each of which serves as a separate light receptor. In some insects, the visual cells of a single ommatidium (right) respond selectively to different colors of light, thus providing the basis for color vision. (Photo courtesy of William H. Amos.)

(2) a transparent crystalline cone, (3) light-sensitive, visual cells arranged in a radial pattern like the sections of an orange, and (4) pigment-containing cells which separate the ommatidium from its neighbors. The location of the pigment-containing cells is such that only light entering the ommatidium parallel (or almost so) to its long axis reaches the visual cells and triggers nerve impulses. Light entering at an angle is absorbed by the screening pigments. Thus each ommatidium is pointed at just a single area in space. If that area is giving off light of sufficient intensity, the ommatidium (and adjacent ommatidia whose field of "view" overlap its own) will respond to it. If not, there will be no response. There may be thousands of ommatidia in a compound eye with their facets arranged over most of the surface of a hemisphere.

A favorite trick of nature photographers is to take a picture of some object as it would be seen though the cornea (all the facets, peeled off together) of a compound eye. Unfortunately, the results (Fig. 26.3) are not at all comparable to what the insect sees. In the photographer's setup, each facet acts as a lens, gathering light rays from all parts of the viewed object and passing them back to the film. As we have seen, however, the presence of screening pigments in the intact compound eye permits light from only one area on the viewed object to reach the visual cells. Thus each ommatidium contributes information about only one area on the object. The other ommatidia contribute information about the other areas. The composite of all the responses of all the ommatidia is a mosaic image—a pattern of light and dark dots which make up the total view.



Fig. 26.3 Portrait of George Washington, as photographed through a portion of the cornea of the compound eye of a water beetle. (Photograph by Prof. Walter E. Flowers, from W. Davis, *Science Picture Parade,* Duell, Sloan and Pearce, 1940.)

Compound eyes probably produce a "grainy" image

The halftone illustrations used in newspapers (and in this book) are created in a similar fashion. If you look carefully at such an illustration (a magnifying lens will help) you will see a regular array of dots of black ink. The differing sizes of the ink dots provide for intermediate shades of gray and thus a rather faithful, but colorless, reproduction of the original scene. The finer the pattern of dots, the better the quality of the illustration. Grasshopper eyes, with relatively few ommatidia, must produce an exceedingly coarse, grainy image. The bee and the dragonfly possess many more ommatidia in their compound eyes and a corresponding improvement in the ability to discriminate ("resolve") detail. Even at that, the resolving ability of the bee eye is poor in comparison with most vertebrate eyes and only 1/60 as good as the human eye.

Because the compound eye does not permit good discrimination of points located close together in space, arthropods are quite nearsighted. Two objects which we could distinguish between at 60 feet could be distinguished by the bee only at a distance of one foot. On the other hand, the compound eye is well-adapted to detect motion. As an object moves across the visual field, ommatidia are progressively turned on and off. Because of the "flicker effect" produced, insects respond far better to moving objects than to stationary ones. Honeybees, for example, will visit windblown flowers more readily than still ones. The ability of the honeybee to perceive motion compares quite favorably with our own.

Some compound eyes become more sensitive in dim light

The quality of the compound-eye image gets even worse in dim light. Those arthropods which are apt to be active in dim light (e.g. crayfish, praying mantis) are able to concentrate the screening pigments of the ommatidia into the ends of the pigment cells. This shift then enables light

entering a single ommatidium at an angle to pass into adjacent ommatidia and stimulate them, too. With many ommatidia responding to a single area in the visual field, the ability to form an image should deteriorate markedly. The praying mantis is probably able to do little more than distinguish light and dark in the evening. The shift in pigments does, however, make it more sensitive to light than it is during the daytime as more ommatidia can detect a given area of light.

Two pigments with different absorption spectra provide the minimum *basis for color discrimination*

Studies of insect behavior show conclusively that some insects, at least, are able to distinguish colors from varying shades of gray. They have true color vision. In order for this to be possible, two or more light-absorbing pigments must be present in the eye, each of which absorbs best at a different wavelength. A single pigment can only provide information about the amount of light given off by an object, that is, its brightness. In such a case, the individual is totally colorblind, with all objects appearing in varying shades of gray. With two or more pigments, however, it becomes possible to distinguish colors because an object giving off rays of predominantly one wavelength will selectively stimulate the receptors containing the pigment which absorbs that wavelength best. Although the actual pigments that permit color vision in certain insects have not been conclusively identified, there is strong evidence for their existence. It has been found, for example, that four of the visual cells in the honeybee ommatidium respond best to yellow-green light (530 nm), two respond maximally to blue light (430 nm), and the remaining two respond best to ultraviolet light (340 nm). This arrangement should enable the honeybee to distinguish colors (except red) and behavioral studies show that this is the case (Fig. 26.4).

Honeybees see ultraviolet light

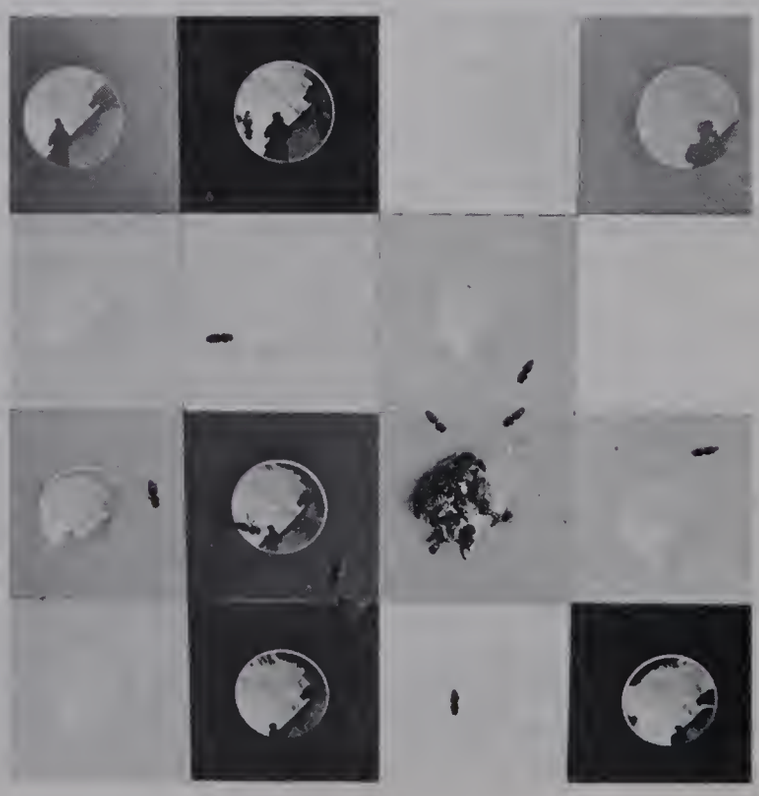


Fig. 26.4 A demonstration of color vision in honeybees. After a period of feeding from a dish placed on blue cardboard, the bees return to an empty dish on a clean blue card. They are able to distinguish the blue card from others of varying shades of gray. (Courtesy of Dr. M. Renner.)

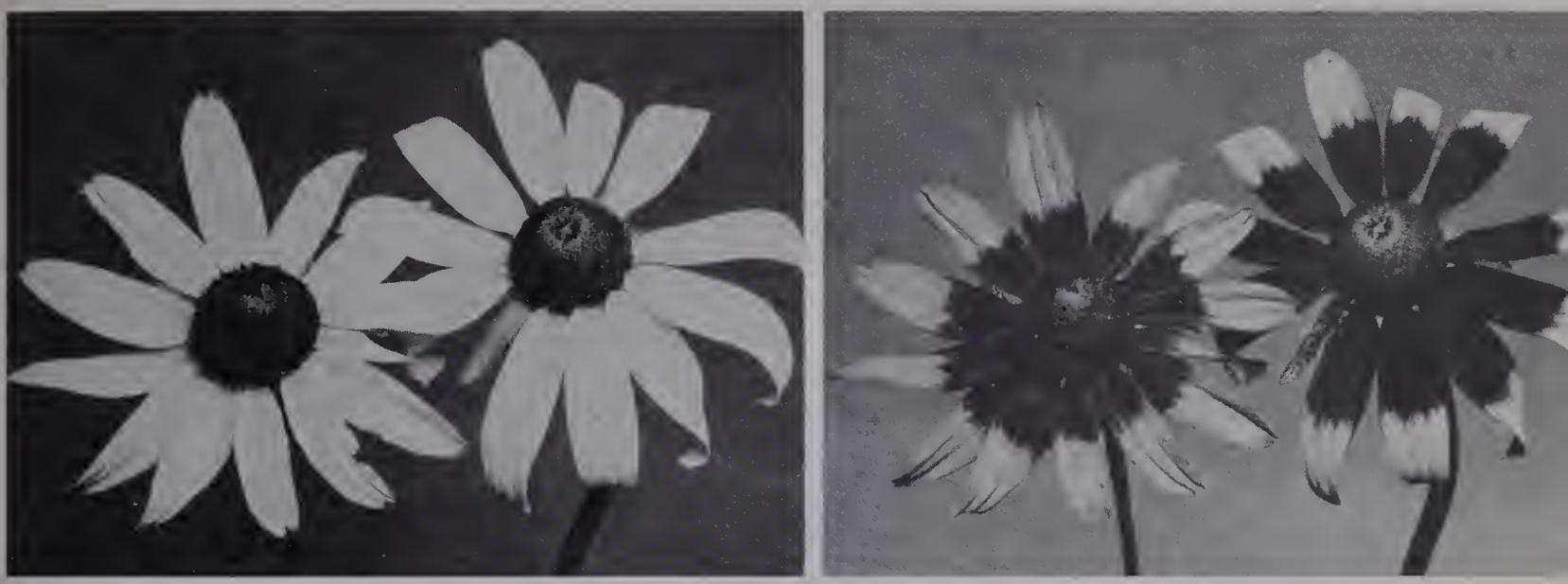


Fig. 26.5 Blackeyed susan photographed in visible light (left) and ultraviolet light (right). (Courtesy of Dr. Thomas Eisner.)

Of what value is ultraviolet vision? Television camera tubes are also sensitive to ultraviolet light, as well as visible light, but the lens with which they are usually equipped is opaque to ultraviolet. (This is why you can't get tanned—or synthesize calciferol—from the sunlight passing through window glass.) However, using a special ultraviolet-transmitting lens, Eisner and his coworkers at Cornell have demonstrated that many insect-pollinated flowers appear to the honeybee quite different from the way they appear to us (Fig. 26.5). The revelation of sharp contrasts between flowers that appear similar to us partly explains the efficiency with which honeybees secure nectar from only one species of flower at a time even when other species are also in bloom. It is also interesting to note that flowers that are pollinated by birds and bats (neither of which have ultraviolet vision) do not have special ultraviolet patterns.

26.3 STRUCTURE OF THE HUMAN EYE

In many respects, the human eye operates like a camera

The eyes of both mollusks and vertebrates operate on the same basic principle as a camera. A single lens focuses light from all parts of the visual field onto a sheet of light-sensitive cells (Fig. 26.6). Despite the close similarity in structure and function between the eyes of mollusks and vertebrates, all the evidence indicates that they arose and evolved quite separately in the two groups.

The human eye is roughly spherical in shape. It is bounded by three distinct layers of tissue (Fig. 26.7). The outer layer, the **sclerotic coat,** is extremely tough. It is white in color (the "white" of the eye) except in the front. Here it forms the transparent **cornea** which admits light into the interior of the eye and bends the light rays so that they can be brought to a focus. The surface of the cornea is kept moist and dust-free by the secretion of the tear glands.

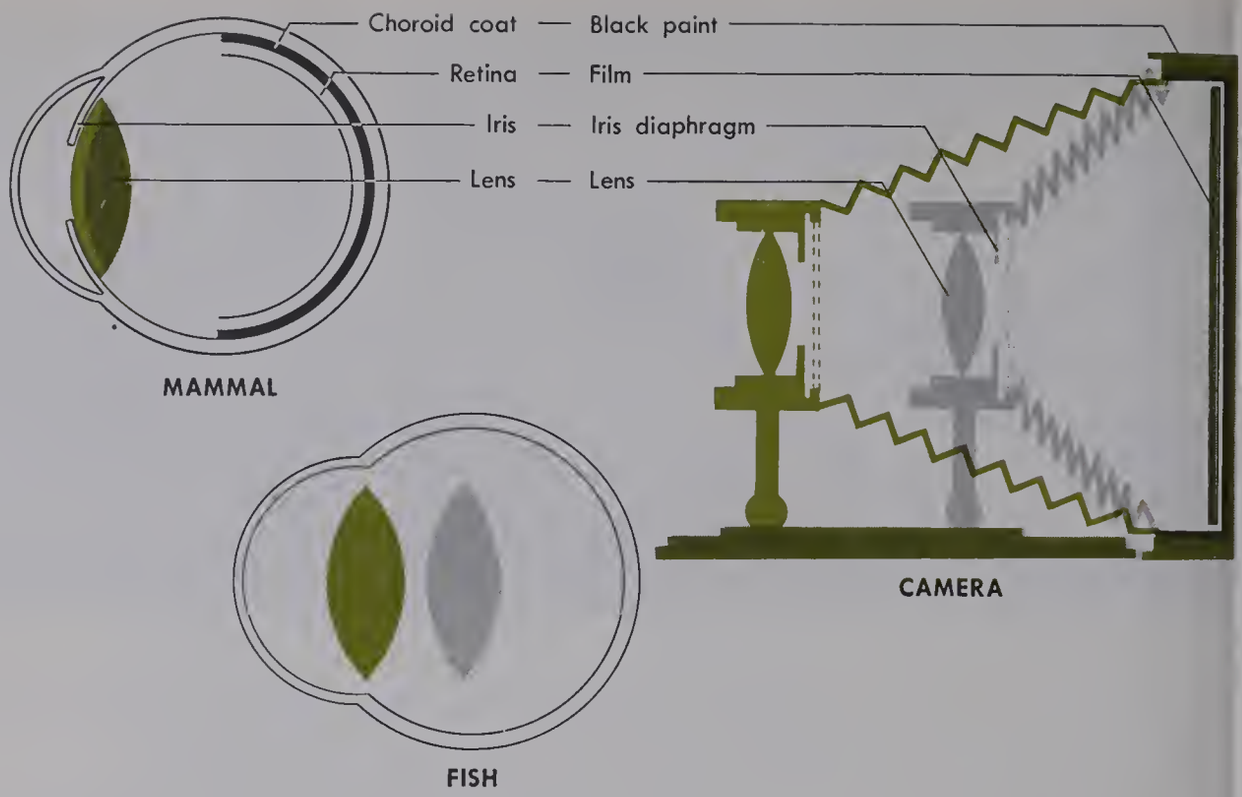


Fig. 26.6 A comparison of two vertebrate eyes and a camera. Each is shown focused on a distant object (gray) and a near object (color). The eyes of amphibians, snakes, and most mollusks work like those of the fish; that is, focus is changed as it is in a camera. Birds and mammals accommodate by changing the curvature of the lens.

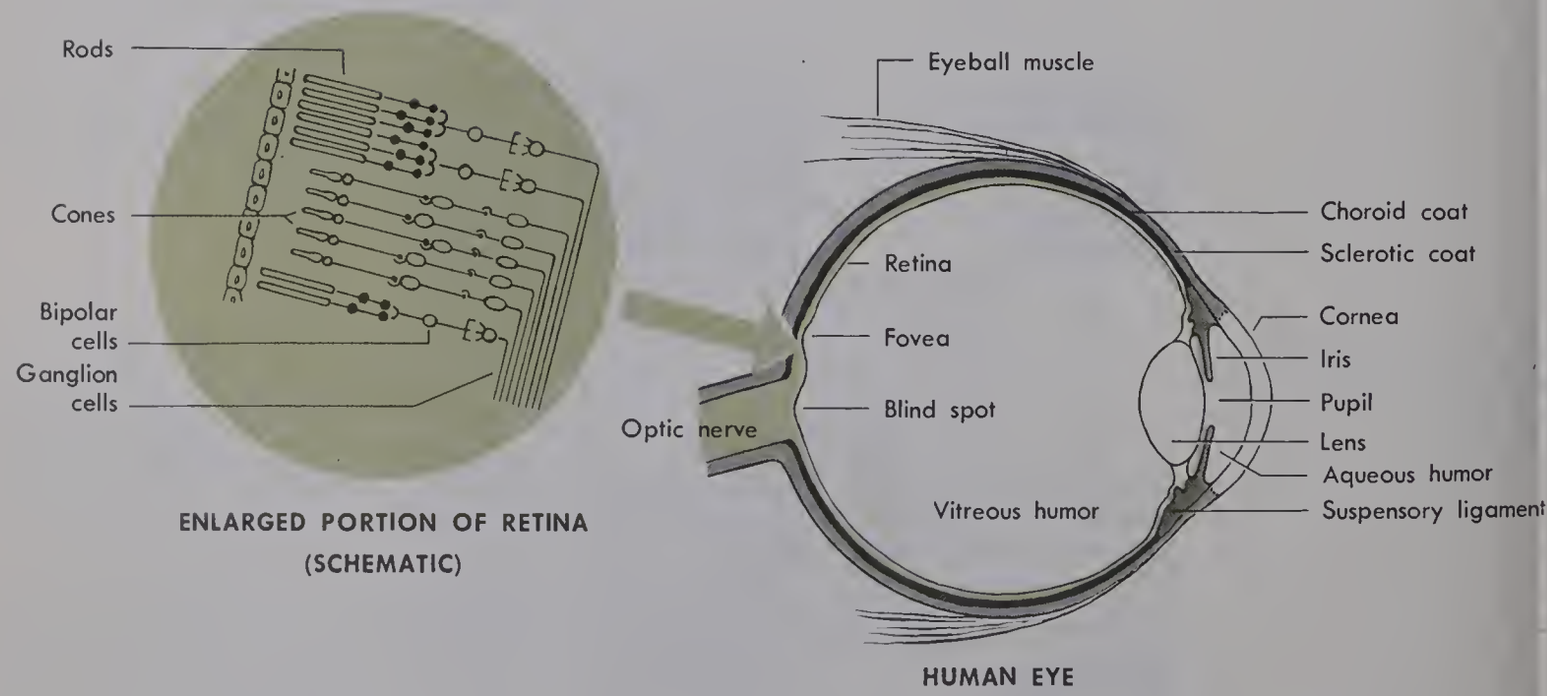


Fig. 26.7 The human eye. The actual light receptors are the rods and cones.

The middle coat of the eye, the **choroid coat,** is deeply pigmented with melanin and well-supplied with blood vessels. It serves the very useful function of stopping the reflection of stray light rays within the eye. This is the same function accomplished by the dull black paint within a camera.

Function of the iris

In the front of the eye, the choroid coat forms the **iris.** This, too, may be pigmented and is responsible for the "color" of the eye. An opening, the pupil, is present in the center of the iris. The size of this opening is variable and under automatic control. In dim light (or times of danger—see Section 25.10) the pupil enlarges, letting more light into the eye. In bright light, the pupil closes down. This not only protects the interior of the eye from excessive illumination, but improves its image-forming ability and depth of field. Photographic enthusiasts, too, make a practice of "stopping down" the iris diaphragm of their cameras to the minimum permitted by the amount of light available in order to get the sharpest possible pictures.

The inner coat of the eye is the **retina.** It contains the actual light receptors, the rods and cones, and thus functions in the same way as the film of a camera.

Accommodation

The **lens** of the eye is located just behind the iris. It is held in position by suspensory ligaments (Fig. 26.7). Ordinarily, these are kept under tension and the lens is correspondingly flattened. However, contraction of muscles attached to these ligaments relaxes them and permits the lens to take on a more nearly spherical shape. These changes in lens shape enable the eye to shift its focus (accommodate) from far objects to near objects and vice versa.

Some people have a difficult time bringing light rays to focus on the retina. If the eyeball is too short, or the lens too flat or too inflexible, the light rays entering the eye will not be brought to a focus by the time they strike the retina (Fig. 26.8). This condition is known as farsightedness because nearby objects are particularly difficult to bring into focus. The use of eyeglasses fitted with convex lenses corrects the condition by helping the eye's own lens to converge light rays more rapidly. Aging people are particularly apt to become farsighted as their lenses become less flexible.

Farsightedness

Nearsightedness

Too long an eyeball or too spherical a lens causes nearsightedness. The image of distant objects is brought to a focus in front of the retina and is out of focus again before the light actually strikes the retina (Fig. 26.8). Nearby objects can be seen more easily. Eyeglasses with concave lenses correct this condition by diverging the light rays somewhat before they enter the eye.

The method of changing focus by changing the shape of the lens has no parallel in photography. Focus is changed in cameras by moving the position of the entire lens with respect to the film. This method is also used in the eyes of some fishes, amphibians, snakes, and some mollusks.

Astigmatism

If either the lens or the cornea has any irregularities in its curvature, all the light rays entering the eye will not be brought to a focus together. This defect is known as **astigmatism.** It may be diagnosed by observing



Fig. 26.8 Common eye defects. The path of light rays without glasses is shown in black; with glasses, in color. The lenses of modern eyeglasses are not so simple in shape as those shown but function in the same way. Irregularities in the cornea or lens of the eye will prevent all the lines in the pattern (center) from appearing equally sharp. This eye defect is known as astigmatism.

a pattern of radial lines as shown in Fig. 26.8). It is corrected by specially ground eyeglasses which compensate for the irregularities.

Cataracts

For reasons which are still obscure, some people develop cloudy areas in one or both lenses. These are called **cataracts** and they cause partial or complete blindness. Today eye surgeons can usually restore vision by removing the defective lenses surgically and giving the patient extra-"strong" eyeglasses to compensate for their loss.

The iris and the lens divide the interior of the eyeball into two main chambers. The anterior one is filled with a watery fluid, the **aqueous humor.** The posterior chamber is filled with a jellylike material of marvelous clarity, the **vitreous humor.**

The eye muscles

Movement of the eyeball is accomplished by three pairs of muscles, the members of each pair working antagonistically. The coordinated action of these muscles enables the eyeball to be rotated in any direction. Thus we are able to train both eyes in a single direction. This produces two slightly differing views of the same scene which our brain is able to fuse into a single, three-dimensional (stereoscopic) image. Improper coordination of the muscles controlling the eye produces such defects as "cross-eyes."

26.4 DETECTION OF LIGHT

The actual visual receptors of the eye are the rods and cones. These are cells which are stacked closely together just beneath the surface of the retina.

Rods provide our maximum sensitivity to light

1. The rods. There are approximately 100 million rods in each eye. They are used chiefly for vision in dim light and are extremely sensitive to light. The image produced by the rods is, however, not a sharp one. Careful microscopic study of the structure of the retina provides an explanation for



Fig. 26.9 Rod cells of a kangaroo rat (25,000 X). The outer segments of the rods contain orderly stacks of unit membranes in which the visual pigment is incorporated. The inner portions contain many mitochondria. The two parts of the rod are connected by a stalk (arrow) that has the same structure as a cilium. (From Porter and Bonneville: *An Introduction to the Fine Structure of Cells and Tissues,* Lea & Febiger, 1964.)

this. The rods function in groups. In other words, a number of rods share a single nerve circuit to the brain. A single rod can initiate an impulse in that circuit but there is no way for the brain to determine which rod in the cluster was involved (Fig. 26.7).

For light to be absorbed, there must be a light-absorbing substance, a pigment. The pigment in the rods is **rhodopsin.** It is incorporated in unit membranes that are neatly stacked in the outer portion of the rod (Fig. 26.9). This arrangement is similar to that which we found in the thylakoids of chloroplast grana (see Section 9.3), another light-absorbing device.

Rhodopsin consists of a protein molecule, opsin, to which is attached a molecule of the carotenoid retinal (formerly called retinene). Retinal itself is light orange, but when attached to opsin, the deep reddish-purple color of rhodopsin is produced. When light is absorbed by rhodopsin, the opposite reaction occurs. The retinal is split from the protein, and the rod becomes partially bleached. At the same time, a nerve impulse is initiated. It has been shown that several steps are involved in the breakdown of rhodopsin. The first is a "light" reaction. The later steps are "dark" reactions, that is, they do not require light. A similar situation occurs in photography. Opening of the camera shutter exposes the film, but no visible image is produced. Only the subsequent chemical changes of the developing process can convert this "latent" image into the visible image of the negative.

Although the rods provide us with a relatively coarse, colorless image, they are extremely sensitive to the presence of light. They are capable of detecting light a billion times less intense than that which our eyes receive on a bright, sunny day. The more light which strikes the rods, however, the more rhodopsin is bleached and the less sensitive the rods become. Fortunately, the process is reversible. Some rhodopsin may be resynthesized directly from its breakdown products, retinal and opsin. There is also evidence that fresh retinal is continually being manufactured in the eye by the oxidation of vitamin A. The body reserves of vitamin A thus provide a large reservoir for the synthesis of retinal. It is no wonder, then, that vitamin A deficiency is usually associated with nightblindness, the inability to see in the dark.

Dark adaptation

In order for the rods to become as sensitive as possible ("dark-adapted") it is necessary that the rate of rhodopsin synthesis exceed the rate of its breakdown. This means that bright light must be excluded from the eye. We all are aware of how difficult it is to see in a dimly lighted room immediately after entering from a brightly lighted one. It takes some thirty minutes in the dark for our eyes to become fully adapted to the dark. For night-flying aviators during the second world-war, the necessity of remaining in the dark for a period before the start of a dangerous mission was nerve-racking indeed. The realization that the rods are insensitive to red light produced a nice solution, however. Red goggles permitted the aviator to carry on normal activity while still enabling his rods to become dark-adapted. The goggles permitted only red light to reach his retina; this stimulated his cones but not his rods.

The cones provide maximum visual acuity and our ability to discriminate colors

2. The cones. We know less about how the cones work than about how the rods work. The cones are especially abundant (about 150,000 in each square millimeter) in a single region of the retina, the **fovea,** a region just opposite the lens. Unlike the rods, the cones operate only in bright light. Furthermore, they enable us to see colors. As we noted in Section 26.2, at least two kinds of cones must be present in order to detect any colors at all. Each must contain a pigment that absorbs a certain wavelength best. Actually, with three kinds of cones, it would be possible to have full color vision if each kind contained a pigment that best absorbed one of the three primary colors: red, green, and blue. Theoretically, the brain could mix three primary color sensations to produce any of the more than 17,000 different hues that the well-trained eye can distinguish. There is good evidence of the presence of a red-absorbing, a green-absorbing, and a blue-absorbing pigment in the fovea. Retinal is the prosthetic group for each of these. It is differences in the protein, opsin, to which the retinal is attached that account for the differences in absorption. The pigments seem to be located in different cones.

A defect in the synthesis of a cone pigment produces color blindness

These discoveries relate nicely to our knowledge of color blindness. As you learned in Section 18.3, red-green color blindness is a sex-linked, recessive trait. Actually, there are two kinds of color-blind people who confuse reds and greens: those lacking the red-absorbing pigment and

those lacking the green-absorbing pigment. Since this is a sex-linked trait, most of the victims in each group are men. Perhaps the recessive gene on their *X* chromosome is coding for a defective opsin and hence a functional red- or green-absorbing pigment is not produced. There is good evidence that the gene for each of these pigments is located at a separate locus on the *X* chromosome.

Far rarer is the case of color blindness involving an absence of the blue-absorbing pigment. The few cases that have been found involve women almost as often as men, so the defective gene is probably located on an autosome. Furthermore, this gene appears to be inherited as a dominant.

In addition to providing the basis for color vision, cones provide us with our most acute vision. The number of cones sharing a circuit to the brain is far fewer than is the case for the rods. Furthermore, the cones are very densely packed in the fovea. Other tissues, such as blood vessels, are absent from this portion of the retina and thus do not interfere with the reception of a distinct image. The image is, however, distinct (and colorful) over just a small area of view. Our ability to direct our eyes quickly to anything in view that interests us tends to make us forget just how poor our peripheral vision is.

The optic nerves are made up of the axons of ganglion cells

All the nerve impulses generated by the rods and cones travel back to the brain by way of neurons in the **optic nerve.** At the point on the retina where the approximately one million neurons converge on the optic nerve, there are no rods or cones at all (Fig. 26.7). This spot, the blind spot, is thus insensitive to light. With the marks in Fig. 26.10 you can demonstrate the presence of the blind spot for yourself. The blind spots of our two eyes do not receive the same portions of the visual image, so that each eye compensates for the blind spot of the other.

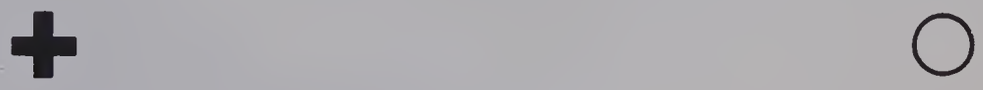


Fig. 26.10 A demonstration of the blind spot. Cover your left eye with your hand and, holding the book at arm's length, stare at the cross with your right eye. What happens to the circle as you slowly move the book toward you?

26.5 HEAT RECEPTORS

Heat is electromagnetic radiation of wavelengths longer than those of light (Fig. 26.1). The human skin is very sensitive to gain or loss of heat. Distributed discretely through the skin are receptors that, when warmed, give rise to the sensation of warmth. Other receptors give rise to the sensation of cold when they are appropriately stimulated. The location of these two classes of receptors can be mapped by using blunt metal probes dipped in hot water and a salt-ice-water mixture respectively. The skin is richly

Our skin has receptors that respond to changes *in temperature*

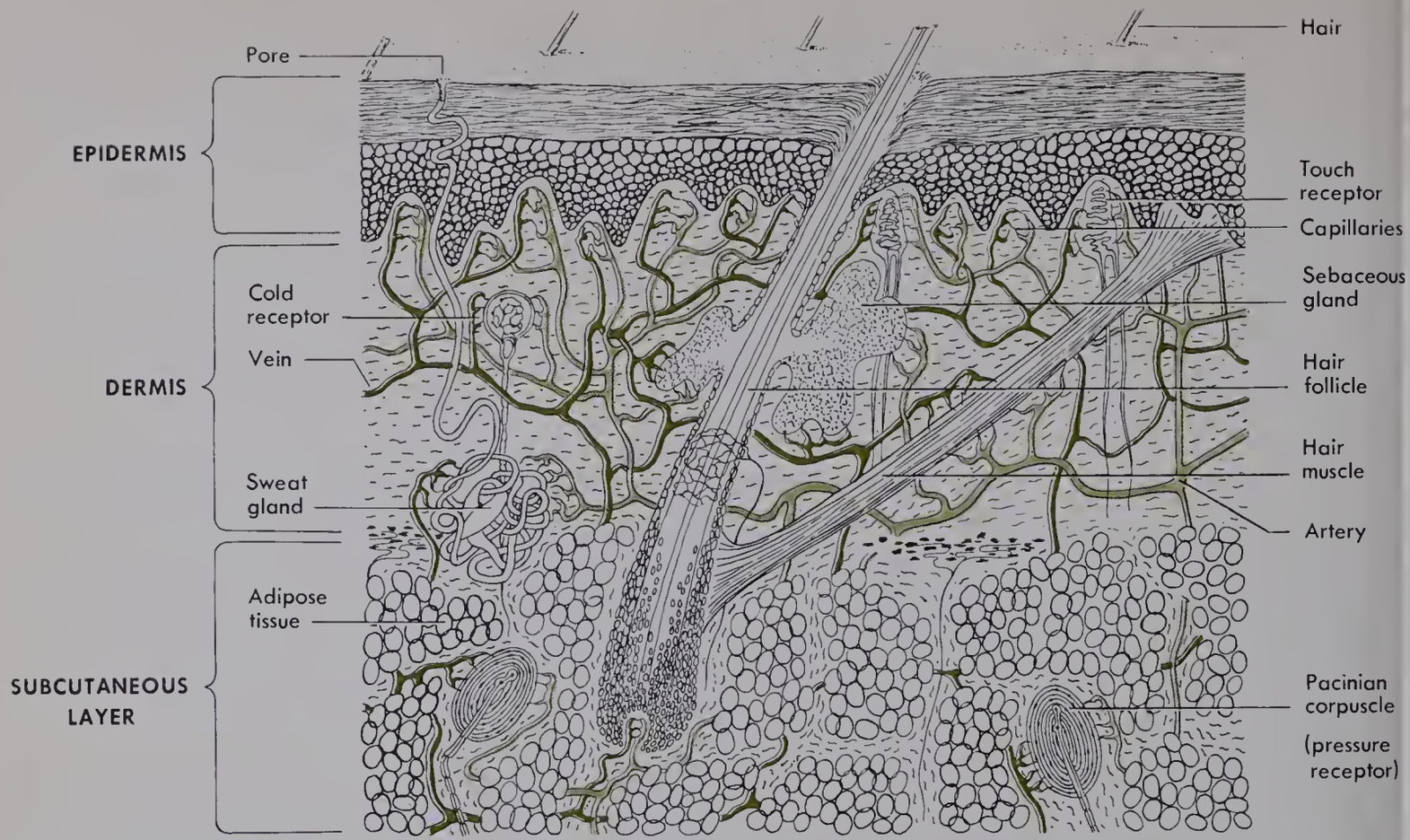


Fig. 26.11 Human skin and its sense receptors. Stimulation of the various receptors shown *probably* gives rise to the sensations indicated for each.



Fig. 26.12 The western diamondback rattlesnake. Note the sense receptors concentrated in the head. The eyes respond to visible radiation. The pits, which are located below the nostrils, detect infrared (heat) radiation. The tongue samples the air for molecules of odorous substances. (Courtesy of New York Zoological Society.)

supplied with sensory neurons, many of which are connected to specialized receptor structures (Fig. 26.11). However, it has not yet been possible to show with certainty whether any of these are exclusively responsible for detecting changes in temperature.

Some snakes contain remarkably sensitive heat receptors located in two pits on the face. These so-called pit vipers include the rattlesnakes (Fig. 26.12), cottonmouth, and copperhead found in this country. The receptors aid the snakes in detecting warm-blooded prey in the dark. Rattlesnakes can strike accurately at a mouse in complete darkness.

The hypothalamus monitors the temperature of the blood

The human body also has receptors that detect internal temperature changes. Two sensitive "thermostats" are located in the hypothalamus (Fig. 27.8) of the brain. The receptor cells in one of these respond to small (0.01°C) increases in the temperature of the blood. As a result of their response, all the activities by which the body cools itself (dilation of the blood vessels in the skin, sweating, etc.) are brought into play when the temperature of the blood begins to rise. It is this temperature-detecting center which enables us to maintain a constant body temperature (homeothermy) during periods of exertion and/or high environmental temperatures. A second receptor in the hypothalamus maintains homeothermy when the body is exposed to chilling. The action of the skin temperature receptors supplements, but cannot replace, the action of the temperature receptors in the hypothalamus.

MECHANORECEPTORS

A wide variety of receptors of mechanical stimuli are found in animals. Each initiates nerve impulses when it is physically deformed by an outside force.

26.6 TOUCH AND PRESSURE

In man, light **touch** is detected by receptors located close to the surface of the skin. These are often found next to a hair follicle (Fig. 26.11). Even if the skin is not touched directly, movement of the hair is detected by the receptor.

Touch receptors

Touch receptors are not evenly distributed over the surface of the body. The skin of the fingertips may contain as many as 100 per square centimeter and the tip of the tongue is similarly well supplied. The concentration of touch receptors in other locations is apt to be much lower. The back of the hand, for example, has fewer than 10 per square centimeter. The exact location of touch receptors can be determined by gently touching the skin with a stiff bristle and marking those spots where the subject detects a distinct touch. An interesting variation on this technique can be carried out with a pair of dividers such as are used in mechanical drawing. With a blindfolded subject, determine the minimum separation of the

points which will give rise to two separate touch sensations. You will find that the subject's ability to discriminate the two points is far better on the fingertips than on, say, the small of the back.

The Pacinian corpuscle is a transducer that converts mechanical energy into electrical energy

Lying deep in the skin are the Pacinian corpuscles (Fig. 26.11). These respond to **pressure.** The mechanism of action of the Pacinian corpuscle has been studied very carefully. It has been found that any mechanical deformation of the corpuscle (such as would be produced by pressure) creates a tiny current of electricity in the sensory neuron originating within it. The greater the deformation of the corpuscle, the greater this "generator current." If the generator current becomes sufficiently large, it initiates a nerve impulse in the sensory neuron. The **threshold** has been reached.

Pacinian corpuscles are also found scattered throughout the interior of the body. Some of these provide the brain with information on the movement of internal organs. We rarely become conscious of this information.

Other pressure receptors exist in the walls of the aorta and carotid arteries. When stimulated by high blood pressure, these initiate nerve impulses which ultimately bring about an inhibition of the heartbeat (see Section 13.19).

Unlike most receptors, proprioceptors do not fatigue

Muscular coordination is aided by the **proprioceptors.** These are sense receptors which are distributed throughout skeletal muscle and tendons. Stretching or contraction of muscles triggers these receptors to initiate nerve impulses. These, in turn, enable the brain to determine the state of contraction of the muscle. If one starts to lose his balance, the brain is informed by the proprioceptors of the legs and corrective action is taken at once. Complex muscular movements, such as are involved in typing, catching a ball, playing the violin, etc., would be impossible without the proprioceptors. The properly timed, coordinated action of a variety of muscles requires that the brain be continually informed of the performance of each. If you ever have had one or both legs "go to sleep," you have some idea of how difficult locomotion would be without proprioceptors.

The mechanism of pain is not yet understood

Massive mechanical stimulation of the skin produces the sensation of pain. Excessive heat, cold, and certain chemicals do this also. The sensation may be produced by the stimulation of a network of nerve fibers in the skin that are not attached to specialized stimulus detectors and thus do not respond unless the stimulus is very strong. On the other hand, pain may be felt as a result of a change in the frequency and pattern of the signals passed to the central nervous system by the specialized skin receptors of touch, pressure, heat, and cold. Perhaps both mechanisms participate in the process.

26.7 HEARING

The ability to hear is the ability to detect mechanical vibrations which we call sound. Under most circumstances, these vibrations reach us through the air. The external ear (Fig. 26.13) may aid somewhat in concentrating sound waves. These then pass down the auditory canal and strike the

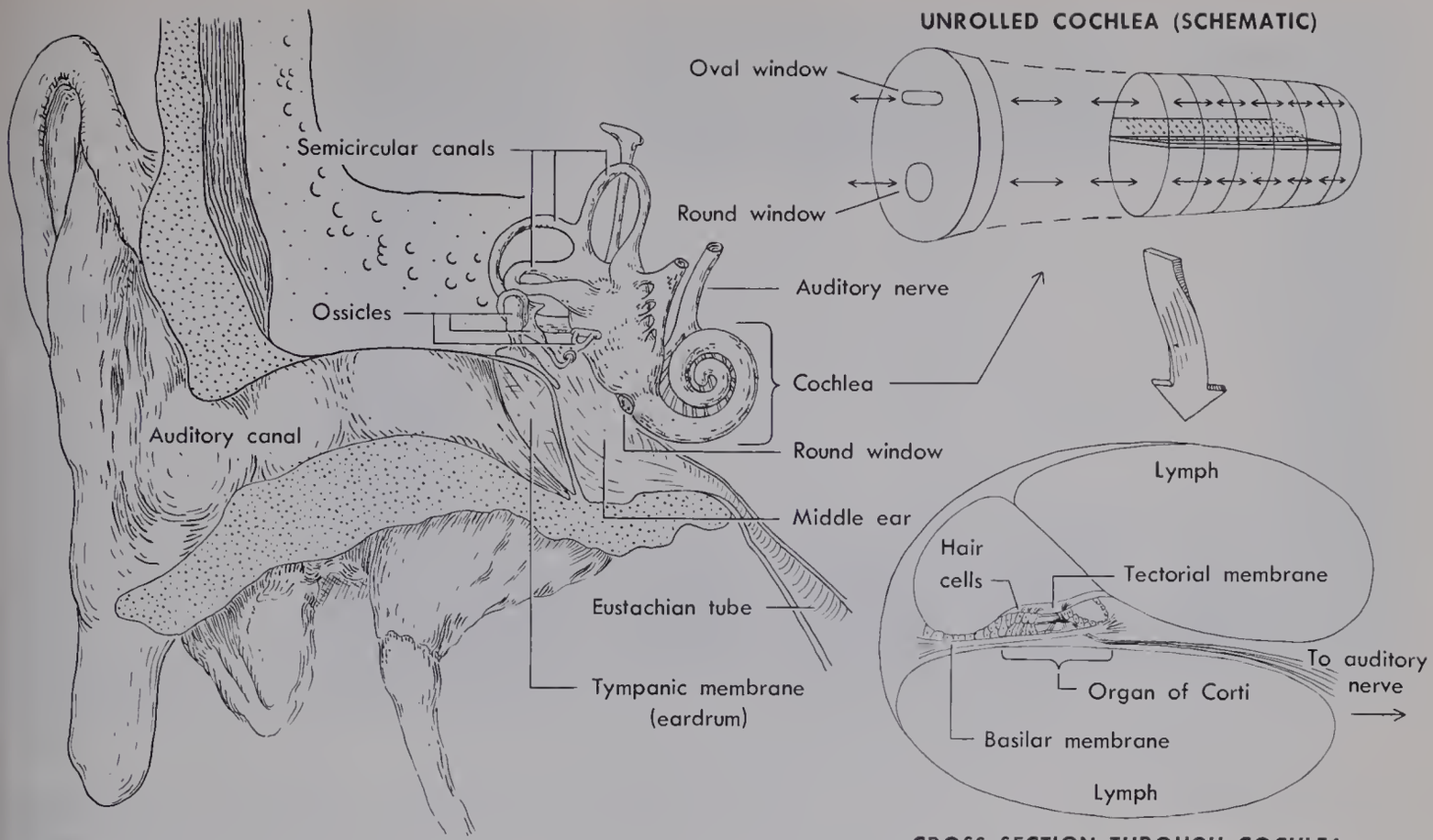


Fig. 26.13 The human ear.

eardrum or **tympanic membrane,** causing it to vibrate. The vibrations of the tympanic membrane are transmitted across the middle ear by three tiny, linked bones, the **ossicles,** which also serve to concentrate the vibrations.

The middle ear

The middle ear is filled with air and is connected to the outside air by means of a **Eustachian tube** opening into the nasopharynx. This opening permits the air pressure on both sides of the tympanic membrane to be kept equal. The "popping" of our ears that we feel when we rapidly change altitude in an unpressurized airplane or an elevator results from the sudden equalizing of pressure when the Eustachian tubes open during swallowing or yawning. Victims of head colds may have inflamed Eustachian tubes which are temporarily prevented from opening. Change in altitude may be very painful at such times because of unequal pressure against the tympanic membranes.

The inner ear

Mechanical vibration of the innermost ossicle, the stirrup, is transmitted through a flexible membrane (the oval window) to the **cochlea** of the inner ear. The cochlea is a tube, about 3 cm in length, which is coiled like a snail shell (Fig. 26.13) and filled with lymph. Running through the cochlea for almost its entire length is a plate of bone and an inner tube which is also filled with lymph. These structures divide the outer tube of the cochlea into two separate chambers. Vibrations of the oval window are transmitted to the fluid in these outer chambers. Because liquids are practically incompressible, it is necessary to have some way of relieving the

pressures created when the oval window is pushed in and out. The flexible *round* window accomplishes this by moving the opposite way (Fig. 26.13).

The organ of Corti

Lying within the inner, or middle, chamber of the cochlea is the **organ of Corti.** It contains thousands of sensitive "hair" cells which are the actual vibration receptors. They are located between the basilar and tectorial membranes (Fig. 26.13). Vibrations in the cochlear fluid cause vibrations in the basilar membrane. This moves the sensitive hair cells against the tectorial membrane, thus stimulating them. Electrical impulses arising in these cells then initiate nerve impulses which travel back along the **auditory nerve** to the brain.

The ear is a remarkably precise and versatile sense receptor. Many people, especially when young, can hear sounds with frequencies (pitches) from as low as 16 to as high as 20,000 cycles per second (hertz). Furthermore, the ear can detect sounds over a wide range of intensities. The loudest sound we can hear comfortably is over one trillion times as loud as the faintest we can detect. The faintest sound is so faint that if our ears were any more sensitive, we would probably detect the sound of random molecular collisions (Brownian movement) within the ear. The power of discrimination of frequencies is also great. A trained musician can distinguish about 15,000 pitches.

cycles/sec = hertz

Pitch discrimination

The way in which the organ of Corti discriminates among different pitches is now fairly well understood. At first glance, it might seem appropriate for the hair cells to send impulses back to the brain at the same frequency as the sound. Something of this sort may actually occur at very low frequencies. It could not occur at frequencies greater than about 1000 hertz because, as we shall see in the next chapter, sensory neurons cannot conduct impulses any faster than that. Actually, even before this limit is reached, the basilar membrane and hair cells begin to respond selectively to the sound frequencies. Low frequencies stimulate the area of the organ of Corti nearest its tip. High frequencies are detected near its base. The intermediate frequencies are detected in an orderly, progressive fashion from one end of the organ of Corti to the other. Evidence to support this view has been obtained by exposing laboratory animals to very intense, pure tones. Eventually the animals become deaf to these frequencies although their ability to hear other pitches is not impaired. In every case, examination of the organ of Corti reveals destroyed hair cells in a single area whose location can easily be correlated with the pitch of the destructive sound.

Bats hear frequencies as high as 150,000 hertz

The hearing ability of bats is extraordinary. The research of zoologist Donald Griffin has shown that bats can hear frequencies as high as 150,000 hertz. Sound at such ultrasonic frequencies travels in fairly straight lines. Bats, flying in complete darkness, are able to locate obstacles (Fig. 26.14) and even insect prey by emitting pulses of this ultrasonic sound and then adjusting their course of flight to the echo which returns to their ears. Such a system of echo-location works on the same principle as the underwater sonar devices developed for submarine detection during World War II.

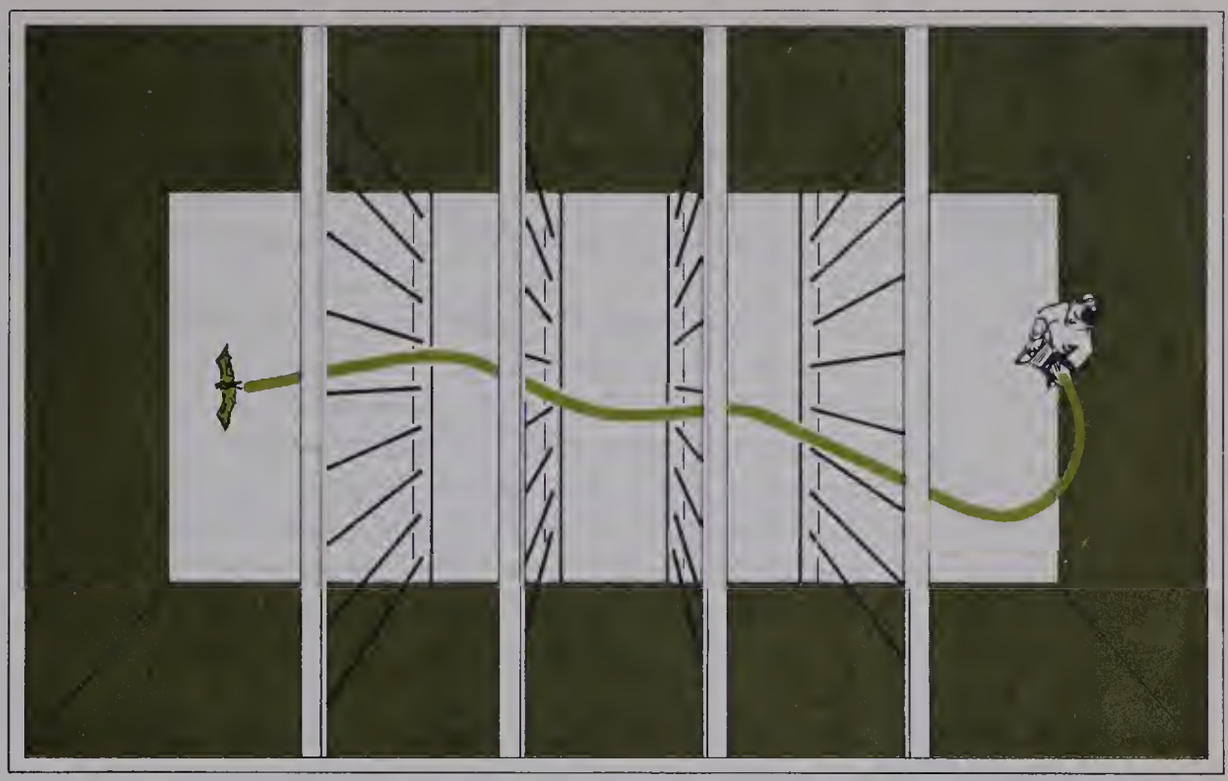


Fig. 26.14 Echo location in the bat. A blindfolded bat can fly between the wires, touching them only rarely. A bat whose ears are plugged collides repeatedly with the wires.

Hearing plays an important part in the lives of other animals, too. Avoidance of predators, the location and courtship of a mate, and the staking out of territorial "claims" may all involve the detection of sounds.

26.8 EQUILIBRIUM

The inner ear also detects: (1) the position of the body with respect to gravity and (2) the motion of the body. These two functions are quite distinct from its function in sound detection and rather distinct from each other. Just above the cochlea are two interconnecting, lymph-filled sacs (Fig. 26.13). These are lined with hair cells which, in turn, are connected to sensory neurons. Attached to the hairs of the hair cells are tiny spheres of calcium carbonate. These are acted upon by gravity and deflect the hairs downward. As the body (or at least the head) is oriented in different directions, the "ear stones" shift their position. The nerve impulses that are initiated by the hair cells are sent back to the brain and inform it of the change.

The function of statocysts

Analogous (but not homologous) structures, called **statocysts,** are found in many aquatic invertebrates. In the crayfish, these hair-lined sacs contain tiny grains of sand instead of ear stones. When the crayfish sheds its exoskeleton, it also sheds its statocysts. As soon as the new exoskeleton is hardened, however, the crayfish seeks out grains of sand to place within its new statocysts. If iron filings are offered to the crayfish instead of sand, it will use these. When the job is complete, a few minutes of experimenta-

tion with a strong magnet will vividly demonstrate the action of the statocysts in maintaining balance. Placing the magnet just above the crayfish draws the iron filings upwards. This, in turn, triggers receptors which would normally be affected only if the animal were upside down. Consequently, the crayfish does, in fact, turn itself upside down in response to the erroneous information received by its central nervous system.

Motion of the human body is detected in the three semicircular canals at the top of each inner ear (Fig. 26.13). These are three fluid-filled tubes, each one of which is oriented in one of the three planes of space. At one end of each canal is a small chamber containing sensory hair cells. Whenever the head is moved, the semicircular canals move, too. The fluid within lags in its motion, however, and consequently there is relative motion between the walls of the canals and the fluid. This motion stimulates the hair cells to send impulses back to the brain. The maintenance of proper balance during athletic activity would be practically impossible without this mechanism. When the hair cells are stimulated in unfamiliar ways, as may occur in a boat or aircraft during rough weather, motion sickness can occur.

CHEMORECEPTORS

Man's receptors of chemicals in the external environment are the taste buds, located principally on the tongue, and the olfactory epithelia, located high in the nasal cavity.

26.9 TASTE

In order for a substance to be tasted, it must be soluble in the moisture of the mouth. Only when in solution can it then stimulate the **taste buds.** Four types of these can be distinguished morphologically. Most of them are located on the surface of the tongue although a few are found on the soft palate, high in the back of the mouth.

Most experimenters agree that there are only four primary taste sensations: sweet, sour, salty, and bitter. By using dilute solutions of sucrose, hydrochloric acid, sodium chloride, and quinine sulfate, respectively, one finds that each of the four primary tastes is localized in a special area of the tongue (Fig. 26.15). However, mapping experiments of this sort also show considerable overlapping of taste areas and considerable variation from one person to the next.

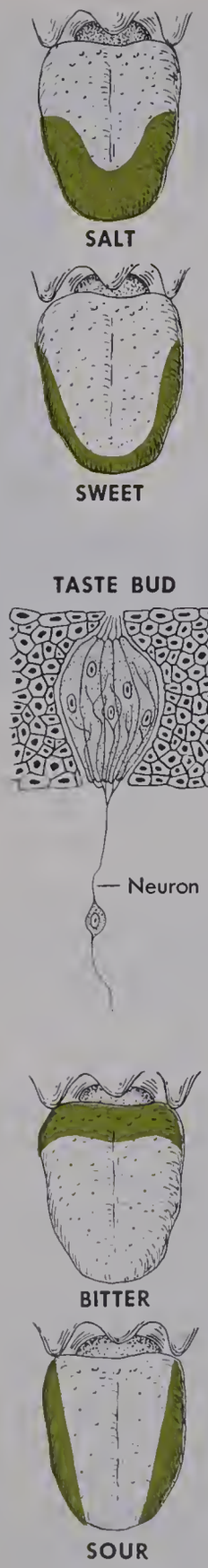


Fig. 26.15 The sense of taste is initiated in the taste buds of the tongue. When stimulated by dissolved chemicals, each initiates one of the four primary taste sensations. While the distribution of taste areas shown is typical, there is considerable variation from person to person.

The existence of four kinds of taste buds and four primary tastes suggests that each type of bud is responsible for one specific taste. With the possible exception of the bitter taste, however, there seems to be no correlation between bud type and the taste detected.

You may well argue that you can detect more than just four tastes. So you can, but this involves other factors. First of all, combinations of the four primary tastes produce new tastes. More important is the role which smell, temperature, and touch receptors play in the tasting process. As one chews food, vapors probably escape through the oral pharynx into the nasal cavity and are detected by our odor receptors. The marked loss of taste which we experience when our nasal cavities are plugged with mucus during a cold supports this point. You can demonstrate it vividly under more pleasant circumstances by applying water, in which two or three cloves have been boiled, to the tongue of a blindfolded subject. He will taste the mixture readily when his nasal passages are open. When holding his nose, however, he will have a very difficult time distinguishing the clove solution from plain water. The temperature and texture of food also play an important part in our sensation of taste.

Influence of smell on taste

Many insects have a well-developed sense of taste. The red admiral butterfly can taste a 0.000078-molar solution of sucrose, which is far too dilute for us to taste. Its taste receptors are located on its legs. Other insects have taste receptors on their antennae and mouth parts.

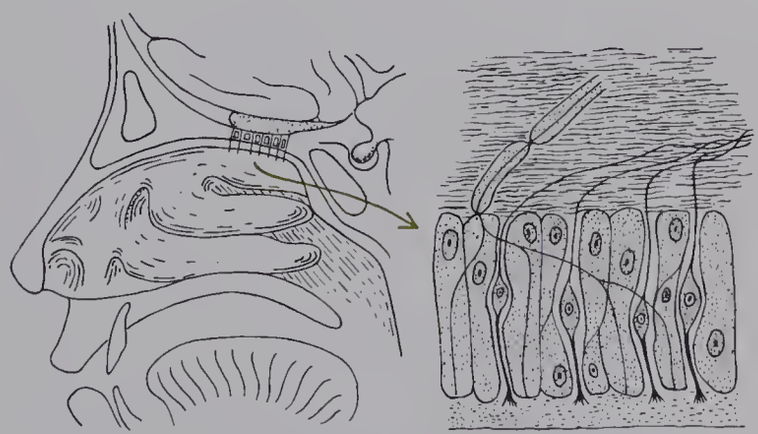


Fig. 26.16 Left: The sense of smell originates in the olfactory epithelium high in the nasal cavity. Right: Although only two kinds of receptor cells can be distinguished, we are capable of discriminating between a wide variety of odorous substances.

26.10 SMELL

Man detects odors by means of receptor cells located in the two **olfactory epithelia** high in the nasal cavity (Fig. 26.16). Each of these areas is about the size of a postage stamp, 250 mm^2. Air drawn in through the nostrils passes over them. Water- and fat-soluble molecules present in the air dissolve in the mucus layer covering the epithelia and give rise to sensations of smell. Vigorous sniffing improves the exposure of the olfactory epithelia to airborne substances.

It is customary to consider man's sense of smell one of his poorest senses. It is true that the sensitivity and the power of discrimination (the ability to distinguish between similar odors) of such animals as the dog and

We sense only one odor at a time

deer are somewhat better than that of man. Nevertheless, man is able to detect a virtually unlimited variety of odors (but just one at a time!) and in many cases at very low thresholds. He can, for example, detect as little as 0.0000000002 gm of vanillin (the active ingredient in vanilla flavoring) vaporized in 1000 liters of air.

The mechanism by which we detect odors is still uncertain

The mechanism by which we are able to detect such a great variety of different odors has puzzled scientists for a long time. Only two kinds of receptor cells can be distinguished in the olfactory epithelium by their *structure*. It seems likely, though, that several (perhaps seven) can be distinguished by their *function*. According to one theory, each of these seven kinds of receptors responds to molecules of a particular class. In most cases, the shape of the molecule determines what class it is in and thus to which receptor it will become temporarily attached. Each class of molecules produces a *primary* odor such as musky, pepperminty, pungent, etc. Complex odors arise when the molecules have a shape that permits them to attach to more than one receptor. They can also arise when a variety of molecules are being given off by the odorous substance. Many odors do, in fact, represent the combined effect of a complex array of chemicals. For example, over 100 substances participate in the production of the odor of geraniums.

Part of the explanation for the low regard we have for our sense of smell is that it does not play a very important part in our lives. Other animals, however, depend greatly on smell to enable them to locate mates, locate food, and escape predators. The male silkworm moth can smell the pheromone released by a female moth two or three miles upwind. Its odor receptors, like those of most insects, are located on the antennae.

Chemical receptors in fish

The remarkable ability of Pacific Coast salmon to return, after a span of four or five years in the sea, to the same fresh-water streams in which they were born probably involves the sense of smell (or taste—there really is not much of a distinction between the two for an animal that lives surrounded by water). Odor receptors in the salmon as well as in most bony fishes are located in two small chambers just in front of the eyes. Water enters and leaves each of these chambers through separate openings, the nostrils. It is interesting to realize that nostrils first served an odor-detecting function in our vertebrate ancestors. Only later, when vertebrates became air-breathing, did the nostrils come to be used to take air to and from the lungs.

Snakes and lizards

Snakes and lizards have a well-developed odor (or taste?) receptor organ, Jacobson's organ, located in the roof of the mouth. They alternately stick their tongue out into the air and then into their Jacobson's organ. Thus they "taste" the air and detect the presence of odors (look back at Fig. 26.12).

26.11 INTERNAL CHEMICAL RECEPTORS

Receptors in the carotid arteries

Man also has receptors that detect chemical changes in the *internal* environment. In the carotid arteries (in addition to the pressure receptors men-

tioned earlier) are cells which are sensitive to increased concentrations of carbon dioxide and other cells that detect decreased concentrations of oxygen. When stimulated, both types initiate nerve impulses that eventually increase the rate of breathing and the rate of heartbeat (see Sections 12.9 and 13.19).

The medulla oblongata

Sensitive carbon-dioxide receptors are also found in the medulla oblongata. These initiate nerve impulses controlling rate and depth of breathing. They provide our most precise control over this function. (See Section 12.9).

The hypothalamus

Our sensation of **thirst** arises as a result of stimulation of special cells in the hypothalamus of the brain. These cells are remarkably sensitive to changes in the osmotic pressure of the blood. If this increases (through loss of water or extra intake of salts), we quickly become conscious of thirst. In addition, vasopressin is released from the posterior lobe of the pituitary gland and acts on the tubules of the kidney to produce maximal reabsorption of water. You remember (see Section 25.9) that it is the hypothalamus itself which manufactures vasopressin for storage in the posterior lobe of the pituitary gland.

Experiments with laboratory rats indicate that they have still other areas in the hypothalamus concerned with sensation. Electrical stimulation of one area causes the rat to feed even though its stomach may be full. Electrical stimulation of another area seems to give the rat a feeling of satiety even though it is actually starving. An increase in the level of blood sugar (glucose) stimulates a similar satiety center in the hypothalamus of dogs.

It would be unwise to draw definite conclusions yet on the relevance of these experiments to man. Even assuming that man has similar hunger and satiety centers in his hypothalamus, these may not actually be the primary stimulus receptors. They may simply receive nerve impulses from receptors located elsewhere in the body. In either case, we have no knowledge of what the actual physical or chemical stimuli are which give rise to these sensations.

26.12 SUMMARY

It should now be clear that man possesses more than just the traditional five senses (touch, taste, smell, vision, and hearing). All of his senses depend upon the presence of specific stimulus receptors. These provide a constant flow of information as to the state of both the external and internal environment. In order to accomplish their functions, all stimulus receptors must possess three general features: (1) they must be so constructed as to have a lower threshold for one type of stimulus than for any other; (2) they must be connected to a sensory neuron; (3) they must be capable of initiating nerve impulses in that neuron.

Properties of sense receptors

Most of our stimulus receptors have a fourth characteristic which is their property of *adapting* quickly to the stimulus. When the stimulus is first applied, the receptor initiates a volley of impulses in the sensory

neuron to which it is attached. With constant exposure to the stimulus, however, the rate of impulse propagation decreases and may eventually cease entirely. This phenomenon is readily apparent in the speed with which we cease to detect an odor to which we are exposed. Sensory adaptation is a useful function in that our nervous system is not continuously bombarded with information about such insignificant matters as the touch and pressure of our clothing. Remember that we defined a stimulus as a *change* in the environment, and it is change that our sense receptors detect. You might well question whether adaptation occurs in vision but, in fact, it does. However, through constant slight, involuntary movements of our eyes, we shift the position of the image on our retinas and thus continue to observe the scene even though no change may have occurred in it. Only stretch receptors such as our proprioceptors and the stretch receptors in the aorta and carotid arteries seem to adapt very little to continuous stimulation.

Sensory adaptation

The brain is the seat of all sensation

It should be stressed again that when conscious sensation occurs, it occurs solely in the brain. All types of stimulus receptors send the same message to the brain: electrochemical impulses in the sensory neurons. It is the brain that assigns meaning to these impulses. A hard blow to the head may exceed the threshold of neurons in the retina and thus trigger nerve impulses in the optic nerve. Although a mechanical force rather than light has given rise to them, the brain still interprets these in the usual way. Consequently, we "see stars."

This principle can be demonstrated more pleasantly with two clean dissecting needles, two lengths of insulated wire, and a dry cell. When the two needles are applied gently to the tongue, a sensation of touch only is felt. When each needle is wired to a terminal of the dry cell, however, a strong taste is detected (Fig. 26.17). The most likely explanation is that electrical stimulation of the taste buds has given rise to these sensations. Can you think of any other possibilities? How could you test them?

The detection of changes in the external environment is just the first step by which nervous responsiveness and coordination are achieved. The next step involves the conduction of nerve impulses from one part of the body to another. The anatomical and physiological mechanisms by which nerve impulses are generated and circuited throughout the body is the topic of the next chapter.

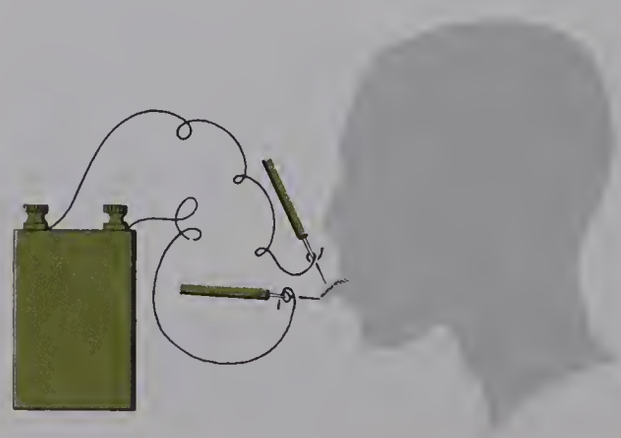


Fig. 26.17 A weak current is detected as a strong taste. When no current is flowing, the dissecting needles are tasteless. What explanation might there be for this?

EXERCISES AND PROBLEMS

1. When trying to see a faint star at night, it is helpful to look slightly away from the spot where the star is. Can you think of an explanation for this?
2. Which of our sense receptors detect events occurring outside of the body? Which detect events occurring within the body?
3. Why does a deficiency of Vitamin A cause night blindness?
4. Why does one's enjoyment of food diminish with a severe cold?
5. Review the different ways in which unit membranes are used in cells.
6. What is a stimulus?
7. Why are we not constantly aware of the touch and pressure of our clothing?
8. What function is common to all sense receptors?
9. In what ways are the compound eye and the human eye similar in function? In what ways are they different?

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27

NERVOUS COORDINATION: THE CONDUCTORS

The Neuron

The Nerve Impulse

The Synapse

The Reflex Arc

The Central Nervous System of Man

The Processing of Visual Information

The Peripheral Nervous System of Man

The Autonomic Nervous System

Drugs and the Nervous System

The ability of animals to respond quickly and in a well-coordinated way to changes in the environment requires more than the presence of stimulus receptors alone. There must also be a system of conductors to transmit information from the receptors to the structures, chiefly muscles and glands, that will take the appropriate action. This system of conductors constitutes the nervous system.

Stimulus receptors generate nerve impulses

All stimulus receptors give rise to nerve impulses. As we shall see, these are basically all alike despite the wide variety of stimuli that initiate them. They are then conducted along an elaborate system of neurons. The route taken by these impulses determines what action is produced. The over-all response of the organism, the coordination of the separate actions participating in this response, and the conscious perception of the stimulus itself all depend upon the circuits traveled by the nerve impulses.

Probably all living cells are capable of detecting stimuli. When an amoeba is pricked with a pin, the entire cell responds to the stimulus. In fact, electrical activity has been detected in the amoeba which resembles that of the nerve impulse in animals.

Animals, with the possible exception of the sponges, have cells that are specialized for conducting electrochemical impulses. The structure, function, and organization of nerve cells are surprisingly similar in the various animals. These cells are called neurons.

27.1 THE NEURON

Structure of the neuron

A neuron is simply a cell that is specialized to conduct electrochemical impulses over a substantial distance. This function is accomplished by means of hairlike cytoplasmic extensions, the nerve fibers (axons). In a large animal such as a horse, these may be as much as several feet in length although only a few micrometers in diameter. They grow out from the **cell body,** which houses the nucleus of the cell. Destruction of the cell body always results in the eventual death of these fibers.

The length of some nerve fibers is so great that it is hard to see how the cell body could exert any kind of metabolic control over them. Nevertheless, there is a steady transport of materials from the cell body along the entire length of the axon. This flow is probably facilitated by the many microtubules (see Section 5.16) present in the cytoplasm within the axon. There is also evidence that the axon receives materials from accessory cells, called Schwann cells. These cells are spaced regularly along the length of the nerve fibers and practically (but not completely) surround them (Fig. 27.1).

In many neurons the nerve impulses are generated in short branched fibers, the **dendrites,** and also in the cell body. The impulses are then *conducted* along a single long fiber, the **axon.** The axon usually branches several times close to its end (Fig. 27.1).

Many axons are covered with a glistening fatty sheath, the **myelin sheath,** which consists of the greatly expanded cell membrane of a surrounding Schwann cell. The membrane is folded around and around the

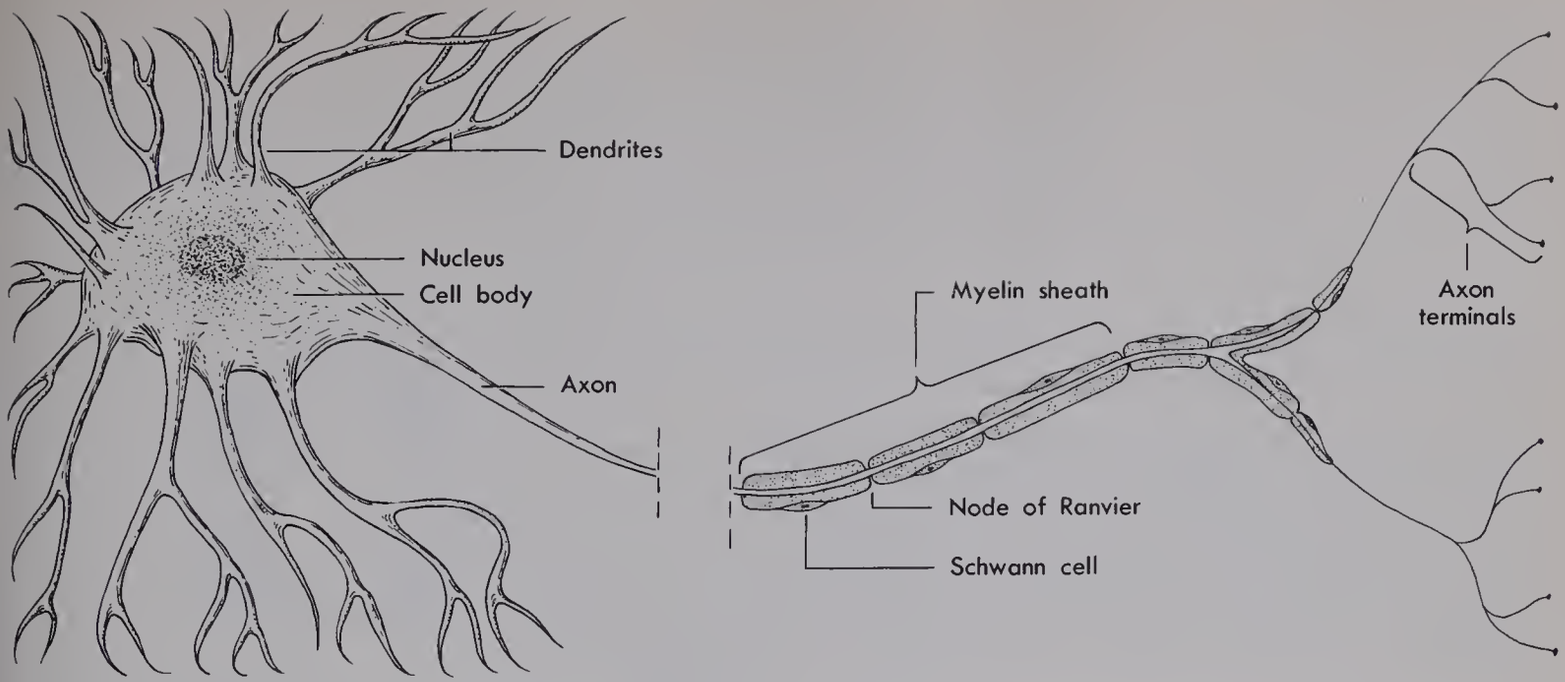


Fig. 27.1 Structure of a motor neuron. Most of the axon has been omitted.

axon to form the sheath. It is believed that the myelin sheath increases the speed with which the nerve impulse passes along the axon. Where the sheath of one Schwann cell meets that of the next, the axon is unprotected. This region, called the node of Ranvier, also seems to play a part in the propagation of the nerve impulse.

Structurally, as well as functionally, neurons can be placed in three distinct groups.

Types of neurons

1. Sensory neurons. Sensory neurons run from the various types of stimulus receptors (discussed in the last chapter) to the central nervous system (brain and spinal cord). The receptors initiate nerve impulses which then travel the length of the sensory neuron. The cell bodies of the sensory neurons are located in clusters **(ganglia)** next to the spinal cord. The axons usually terminate at association neurons.

2. Association neurons. Association neurons are found exclusively within the spinal cord and brain. They are stimulated by impulses which reach them from sensory neurons or from other association neurons.

The association neurons form the intermediate link in the pathway of almost all nervous coordination. The vast number of association neurons in man's central nervous system (several billion at least) and the incredible number of cross connections between them provide us with a virtually limitless number of possible circuits for nerve conduction.

3. Motor neurons. Motor neurons transmit impulses from the central nervous system to the muscles and glands which will bring about the coordinated response of the body. They are usually stimulated by association neurons although, in a few cases, impulses may pass directly from a sensory neuron to a motor neuron.

27.2 THE NERVE IMPULSE

Whatever their differences in appearance, we have no reason to believe that the three types of neurons differ in the nature of the impulses they transmit. However, most of our knowledge of the nerve impulse has come from the study of motor neurons.

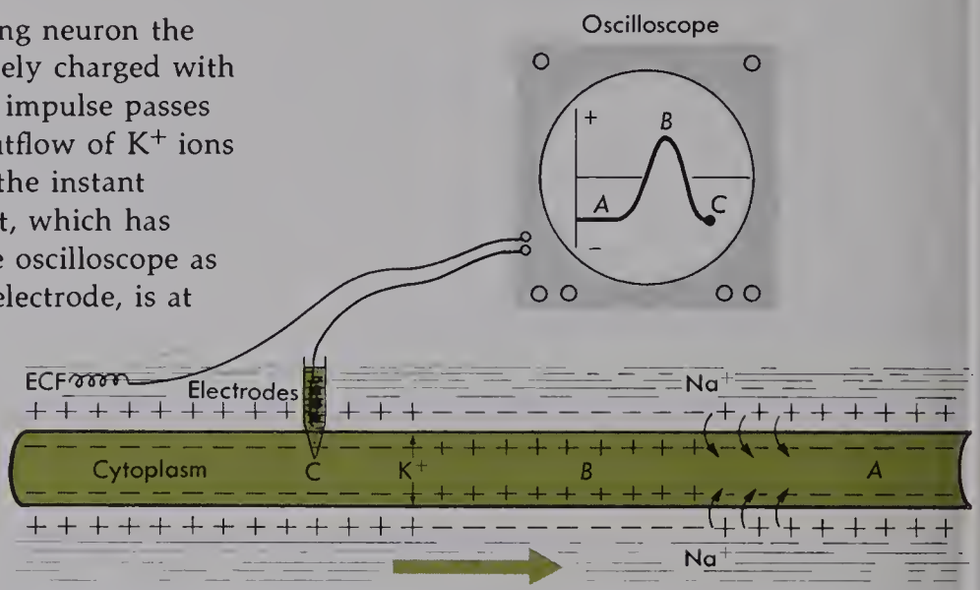


Fig. 27.2 The nerve impulse. In the resting neuron the interior of the axon membrane is negatively charged with respect to the exterior (*A*). As the nerve impulse passes (*B*), the polarity is reversed. Then the outflow of K^+ ions quickly restores normal polarity (*C*). At the instant pictured in this diagram, the moving spot, which has traced these changes on the screen of the oscilloscope as the impulse swept past the intracellular electrode, is at position C.

Active transport establishes a voltage across the cell membrane

With delicate electrodes and sensitive recorders (Fig. 27.2), it is possible to study the electrical properties of the nerve impulse. This equipment reveals that the cytoplasm of the resting neuron is negatively charged with respect to the ECF. The size of this charge across the cell membrane is about 0.07 volt. It exists only so long as the neuron carries on a slow, but unceasing, oxidation of glucose to produce ATP. The energy stored in ATP is used to actively transport Na^+ ions from the interior of the neuron to the ECF and potassium (K^+) ions from the ECF to the interior. This process of active transport leads to a concentration of Na^+ in the ECF ten times as large as that in the cytoplasm and a concentration of K^+ ions in the cytoplasm thirty times that in the ECF. These differences in concentration create a strong tendency for the K^+ ions to diffuse out of the neuron and the Na^+ ions to diffuse into it. The membrane of the resting neuron is virtually impermeable to the passage of Na^+ ions. The K^+ ions do diffuse out, however, and as they do so the interior of the neuron becomes negatively charged with respect to the exterior. When the charge reaches about 0.07 volt, the process reaches equilibrium. The tendency of the K^+ ions to diffuse out because of the concentration gradient is balanced by the electrical attraction of these positively charged ions for the negatively charged interior. There are a variety of stimuli such as a negatively charged electrode, heat, mechanical deformation, and certain chemicals that will increase the membrane's permeability to Na^+ ions and permit them to re-enter the neuron. This, in turn, reduces the voltage between the ECF

and the cytoplasm. If the stimulus is a very weak one, the process does not go too far. The tiny current generated dies out quickly and the normal 0.07-volt polarity is re-established. In such a case, we say that the stimulus was subthreshold. However, if the stimulus is strong enough, the depolarization proceeds to the point where the voltage is reduced to about 0.05 volt. At this value (the **threshold**), the permeability of the membrane to the inflow of Na^+ ions suddenly increases sharply, and Na^+ ions flow in with a rush, eliminating the voltage entirely. In fact, they create a momentary overshoot, with the interior of the membrane now becoming positively charged.

Depolarization

The sudden influx of Na^+ ions at the stimulated point of the membrane has the interesting property of increasing the permeability of adjacent portions of the membrane to Na^+ ions. Consequently, the process is repeated continuously along the length of the nerve fiber, each portion of the fiber triggering the depolarization of the portion adjacent to it (Fig. 27.2). The resulting wave of depolarization which sweeps down the fiber is the **nerve impulse.**

Function of the nodes of Ranvier

In myelinated fibers, depolarization seems to occur only at the nodes of Ranvier. However, the depolarization of one node creates at the next node an almost instantaneous *generator current* which leads to *its* depolarization. The nerve impulse thus jumps rapidly from node to node. It is not surprising that myelinated axons conduct nerve impulses more rapidly than nonmyelinated ones.

The firing of a neuron is an all-or-none event

The strength of the nerve impulse is simply a property of the neuron itself. It has nothing to do with the strength of the stimulus. As long as a stimulus just exceeds the threshold of the neuron, the neuron will "fire." Stimuli of greater strength can do no more than this. We say that the response of the neuron is "all-or-none," but it should be noted that strong stimuli may give rise to a greater number of impulses in a given period of time than weak ones.

The nerve impulse should not be compared to the flow of current in a wire. The processes are not at all similar. In the latter case, an electrical impulse is conducted along the wire at the speed of light. In the neuron, an electro*chemical* reaction is simply moving down the length of the neuron.

Even in our fastest neurons, this wave of depolarization proceeds at less than 200 miles/hour. In one sense this is very rapid, but of course it does not begin to compare with the speed of light (186,000 mi/sec).

A better analogy for the nerve impulse is the fuse to a string of firecrackers. When a burning match is applied to the end of the fuse, its threshold is reached and a spark begins to pass along the fuse. The energy for this movement comes from the energy of the chemicals stored in the fuse itself, not from the energy of the burning match. There is no weakening of the spark with distance traveled. If the fuse branches, the spark travels with undiminished vigor along each branch. (Neither of these conditions occurs in electrical circuits.)

The refractory period

A second stimulus applied to a neuron less than 1/1000 sec after the first will not trigger any impulse. The membrane is depolarized and the neuron is said to be in its **refractory period.** In some of our neurons, the refractory period lasts for only 1/1000 to 2/1000 of a second. This means that the neuron can transmit 500–1000 impulses every second. Other neurons, especially those of cold-blooded animals, repolarize more slowly. When the 0.07 volt polarity is re-established, the neuron is ready to "fire" again. Repolarization is established by the rapid diffusion of K^+ ions from within the cell to the ECF. Only when the neuron is finally rested are the Na^+ ions that came in at each impulse actively transported out of the cell. For each one that leaves, a K^+ ion returns, thus maintaining normal polarization.

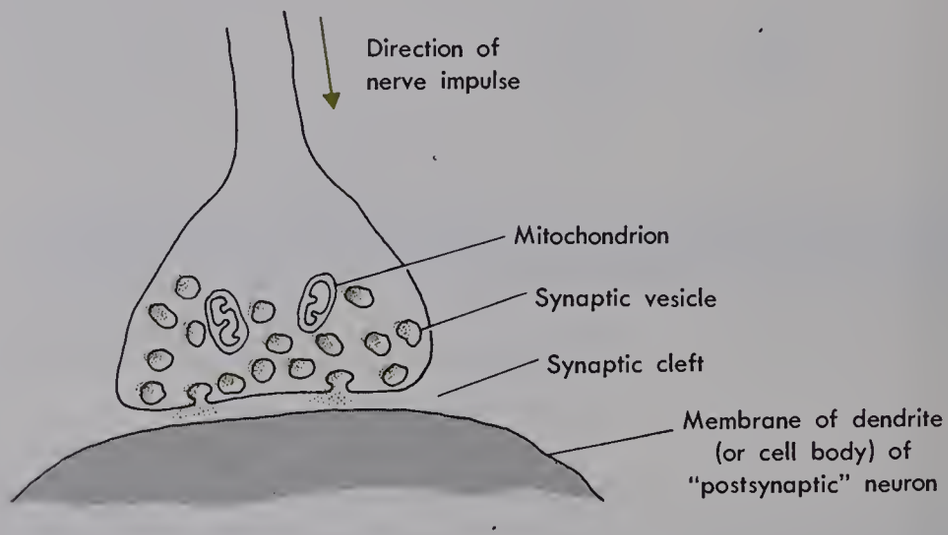


Fig. 27.3 Generalized structure of the synapse. The arrival of an impulse at the synaptic knob causes a vesicle-contained chemical (e.g. acetylcholine) to be discharged into the synaptic cleft. At excitatory synapses, this substance depolarizes the membrane of the postsynaptic neuron. At inhibitory synapses, the substance released hyperpolarizes the postsynaptic neuron. In both cases, enzymes degrade the chemical soon after its release, thus restoring the synapse to its initial state.

27.3 THE SYNAPSE

The points at which the axon terminals of one neuron are in contact with other neurons are called **synapses.** Each axon terminal is swollen to form a synaptic knob (Fig. 27.3). The knob contains a chemical which, when an impulse arrives, is released into the narrow synaptic cleft or gap. At synapses outside the central nervous system, this substance is **acetylcholine** (ACh). Accumulation of ACh in the synaptic gap reduces the polarization of the dendrites or cell body of the next ("postsynaptic") neuron. If the depolarization is sufficient, the threshold of the postsynaptic neuron will be reached and a nerve impulse generated in it. Thus ACh serves as a chemical "transmitter" of the nerve impulse. Because *dendrites* do not release a chemical transmitter, any nerve impulse passing along a single

Action of acetylcholine

The synapse acts as a one-way valve

neuron from axon to dendrites would die out at the synapse. Thus the synapse acts as a valve permitting only one-way flow of nerve impulses.

Chemical transmission of the nerve impulse across the synapse also occurs in the central nervous system but positive identification of the substances involved has been very difficult to achieve. ACh, four amino acids (glutamic acid, glycine, aspartic acid, and gamma aminobutyric acid —GABA), and four amino acid derivatives (noradrenaline, serotonin, dopamine, and histamine) are all likely candidates. Dopamine is an intermediate in the synthesis of noradrenaline from the amino acid tyrosine. Serotonin is synthesized from the amino acid tryptophan. It is closely related to the plant hormone IAA (see Section 24.4).

Other transmitter substances

In view of the structural and functional complexity of the central nervous system, it might be expected that a number of different chemical transmitters would be found. Furthermore, impulses arriving at the synaptic knobs of some association neurons in the central nervous system *inhibit* depolarization of the neurons with which they synapse. The chemical transmitter used in these cases seems to exert its effect by increasing the permeability of the neuron membrane to K^+ ions. As additional K^+ ions diffuse out of the cell, the interior becomes even more negative with respect to the exterior. This *hyper*polarization may reach a value of 0.08 volt. A neuron that is hyperpolarized by the influence of an inhibitory transmitter substance appears to have an increased threshold, that is, the cell is less easily stimulated. Actually, the threshold *voltage* (about 0.05) has not changed. It is simply a matter of whether the depolarization produced by the excitatory synapses *minus* the hyperpolarizing effect of the inhibitory synapses can reach this value or not.

Inhibitory synapses

Of what use are inhibiting neurons? Inhibition of muscles is just as important as stimulation, if coordinated movements are to be made. Imagine trying to catch a ball if all your muscles contracted at once. While *failure* to *stimulate* does result in inhibition, specific inhibitory neurons in the central nervous system undoubtedly provide even more precise control.

Removal of the transmitter substance

The proper operation of the synapse requires that the chemical transmitter be removed from the synaptic gap as soon as it has done its job. If it is not, it will "fire" the neuron over and over again. The removal of ACh is accomplished by the enzyme acetylcholinesterase, which hydrolyzes the molecule into inactive fragments. Substances have been discovered which interfere with the action of acetylcholinesterase. These seriously upset normal nervous activity. Some of them are used as insecticides; others are manufactured for possible use as "nerve gases" in warfare.

The physiology of the synapses in the central nervous system can best be interpreted in terms of the release of a chemical transmitter. It is known, for example, that there is a definite delay (about 0.6 millisecond) in the transmission of a nerve impulse from one neuron to the next. This can be explained by the need for a sufficient amount of the "transmitter" substance to be released to trigger the next neuron. It is also known that synapses present substantial resistance to the continued movement of nerve impulses. A single impulse often dies out at a synapse, but several

Summation of impulses

impulses reaching the synapse within a brief period of time will trigger an impulse in the next neuron. Probably the quantity of "transmitter" substance released by just a single impulse does not depolarize the neuron sufficiently to exceed its threshold.

On the other hand, rapid, repeated use of a given synapse will soon cause it to become fatigued. It presents an increasingly steep barrier to the passage of nerve impulses. This can be interpreted on the basis of a temporary exhaustion of the reserves of "transmitter" substance stored in the synaptic knobs.

27.4 THE REFLEX ARC

In man, the simplest unit of nervous response is the reflex arc. The individual neuron is the unit of structure of the nervous system, but the reflex arc is the unit of function. We can illustrate the reflex arc by examining what goes on when you touch a hot stove and quickly pull your hand away. This response is called a withdrawal reflex. In order for it to be carried out, the following actions take place:

The withdrawal (flexion) reflex

1. The stimulus is detected by receptors in the skin.
2. These initiate nerve impulses in the *sensory* neurons leading from them to the spinal cord.
3. These impulses enter the spinal cord and initiate impulses in one or more *association* neurons.
4. Association neurons initiate impulses in the appropriate *motor* neurons.
5. When these impulses reach the junction between the motor neurons and the muscles, the *muscles* (called *flexors*) are stimulated to contract. Your hand is withdrawn.

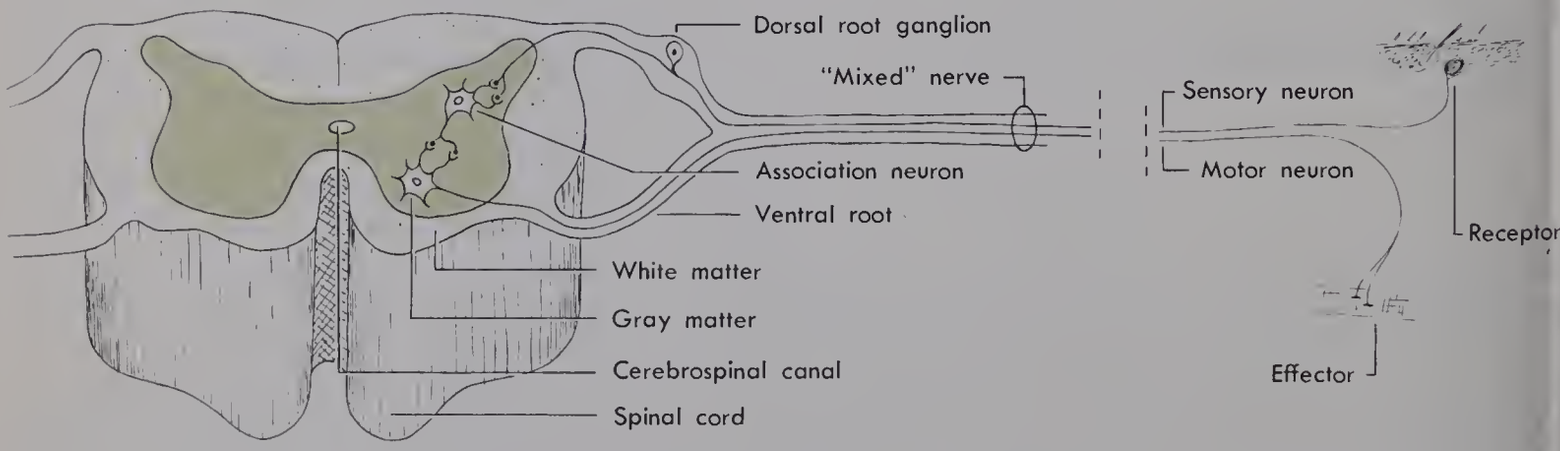


Fig. 27.4 The anatomical basis of the reflex arc. Response to stimuli requires a receptor of the stimulus, which initiates a nerve impulse in a sensory neuron. This impulse usually passes to one or more association neurons, then to a motor neuron which conducts it to the effector. The effector carries out the response. Incoming impulses also pass to *inhibitory* association neurons. These inhibit the motor neurons running to those effectors whose action would interfere with the response.

Reflex inhibition

As mentioned earlier, efficient withdrawal of the hand also requires that some of the muscles in your arm (the *extensors*) be inhibited. This is accomplished by inhibitory association neurons in the spinal cord. When they are stimulated by the sensory neurons, they inhibit the motor neurons running to the extensor muscles.

The structural basis for these actions is shown in Fig. 27.4, although it is a vastly oversimplified representation of what actually takes place. The organization of sensory, association, and motor neurons is not a simple 1:1:1 pattern. The axon terminals of a given sensory neuron form synapses with several association neurons. A given association neuron may, in turn, have hundreds of different axons (both of sensory neurons and other association neurons) converging upon it. A motor neuron may have as many as 5000 different association (and occasionally sensory) neurons converging upon it (Fig. 27.5). Furthermore, as we saw in the previous section, some association neurons may *inhibit* synaptic transmission from other association neurons. It is this multitude of interconnections which

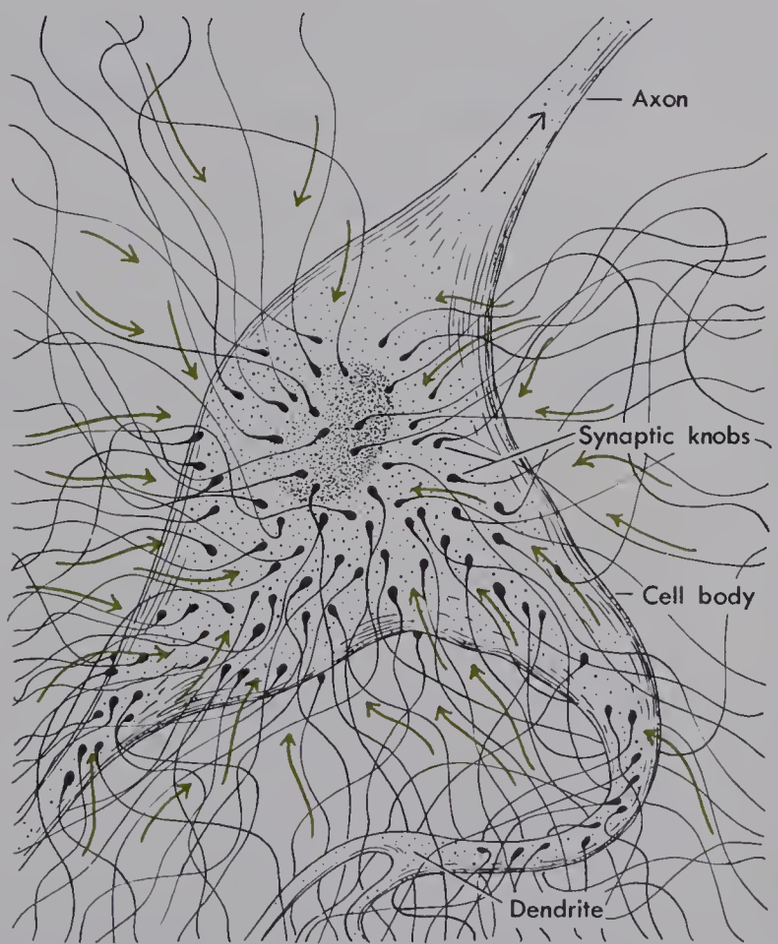


Fig. 27.5 The dendrites and cell body of a single motor neuron in the human may have as many as 5000 association neurons converging on them. Some of these release excitatory substances at the synapse. Others release inhibitory substances. Several excitatory impulses must reach the motor neuron at once in order to initiate a nerve impulse in it. The number must be increased if inhibitory impulses are reaching the motor neuron at the same time.

provide for complex responses and which also enable us to keep informed of what our body is doing. The withdrawal reflex does not require the participation of the brain at all. However, you are aware of what you have done. You can even learn to inhibit your response to the stimulus. These actions would be impossible were it not for other association neurons running up and down the spinal cord informing the brain what is happening and perhaps relaying modifying commands to the motor neurons.

The multitude of interconnections in the central nervous system also provides the basis for variability of response. Many of our complex activities, for example those involved in an athletic maneuver, can be markedly improved with practice. There is improvement in the speed of the response as well as in its precision. This improvement suggests that the nerve impulses are finding ever-shorter, but already existing, routes through the brain and spinal cord. These alternative routes are made possible by the large number of interconnections between sensory, association, and motor neurons.

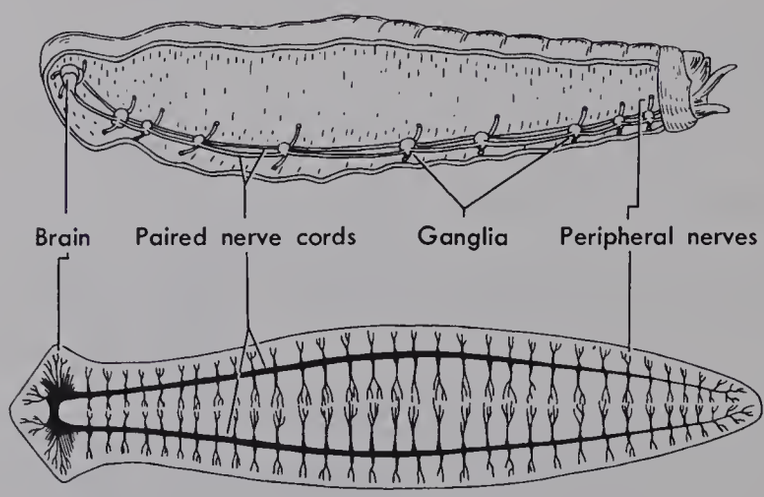


Fig. 27.6 Nervous system of the grasshopper (above) and planarian (below).

Ganglia

In most animals one can distinguish two major divisions of the nervous system. The **central nervous system** of such different forms as planarians, the earthworm, and the grasshopper consists of clusters of cell bodies, the ganglia. Generally the ganglia are located in parts of the body where a good deal of sensory information is being received (e.g. the head) or precise control of muscles (e.g. near the mouth parts) is needed. The ganglia are connected to one another by one or more nerve cords consisting chiefly of the fibers of association neurons (Fig. 27.6). Sensory and motor axons run to and from the ganglia. They are bundled together in cables (nerves) and make up the **peripheral nervous system.** Because most nerves contain both sensory and motor axons, they are called **mixed nerves.**

Mixed nerves

The peripheral nervous system serves to inform the central nervous system of stimuli that have been detected and to cause the muscles and glands to carry out a response. The central nervous system serves as a coordinating center for the actions to be carried out. From what we have

said, it should be clear that neither the central nor the peripheral nervous system can function independently of the other. However, with an organism as complex as man, we will better understand the special features of each system if we study them separately.

THE CENTRAL NERVOUS SYSTEM OF MAN

Our central nervous system consists of the spinal cord and the brain.

27.5 THE SPINAL CORD

The spinal cord is a glistening white cord which runs from the base of the brain down through the backbone. A cross section of the spinal cord reveals that only the outer portion, the "white matter" of the cord, is white. The inner portion is "gray matter." Running vertically through the gray matter is a central canal filled with cerebrospinal fluid; the canal connects with cavities (ventricles) in the brain which are also filled with this fluid.

White matter vs. gray matter

The white matter consists chiefly of long, myelinated nerve fibers running up and down the cord. The gray matter is closely packed with the cell bodies of association and motor neurons.

The dorsal and ventral roots

At evenly spaced intervals along *each* side of the spinal cord are 31 pairs of projections, the nerve **roots.** These unite directly to form the mixed nerves of the peripheral nervous system. All the sensory neurons reaching the spinal cord in a mixed nerve pass into a dorsal root and then into the gray matter of the cord itself. The cell bodies of these sensory neurons are located in ganglia in the dorsal roots (Fig. 27.4). All our motor neurons originating in the spinal cord pass out ventral roots before uniting with the sensory axons to form the mixed nerves.

The separation of sensory and motor axons in the roots is easily demonstrated when they become accidentally cut or otherwise damaged. Destruction of dorsal roots causes a loss of sensation in that part of the body which supplied sensory impulses to the damaged roots. Destruction of ventral roots, on the other hand, causes a muscular paralysis of that part of the body supplied by the motor neurons running through those roots. To achieve substantial anesthesia or paralysis, several adjacent roots must be cut because there is considerable overlapping of function between the mixed nerves they form.

The spinal cord carries out two main functions in nervous coordination. First, it connects the peripheral nervous system to the brain. Information reaching the spinal cord through sensory neurons may be transmitted up the cord by means of association neurons. In the brain all this information can be compared and then appropriate action dictated. Impulses leav-

ing the brain travel back down the cord by way of other association neurons and then leave the cord in the motor neurons.

The association neurons carrying impulses *from* specific receptors or *to* specific effectors are not organized randomly in the spinal cord but are, instead, grouped together in **tracts.** Impulses from the body's pain receptors, temperature receptors, proprioceptors, etc., each pass up the cord in their own special tracts. Impulses passing down the cord to the motor neurons are also localized in tracts. Curiously enough, impulses reaching the cord from the left side of the body eventually pass over to tracts running up to the right side of the brain, and vice versa. In some cases, this "crossing over" occurs as soon as the impulses enter the spinal cord. In other cases, it does not take place until the tracts actually enter the brain.

Crossing over of spinal tracts

The second function of the spinal cord is to act as a minor coordinating center itself. Simple reflex responses, like the withdrawal reflex, can take place through the sole action of the spinal cord. The brain does not need to receive or initiate any nerve impulses for the action to be carried out successfully. Although only relatively simple coordination can be carried out by the spinal cord alone, its actions are a good deal more complex than we have suggested. Even for such a "simple" response as the withdrawal reflex, many motor neurons must be stimulated at the proper moment while other motor neurons are inhibited.

27.6 THE BRAIN

The activity of the brain is even less well understood than the activity of the spinal cord. Basically, the brain receives nerve impulses from the spinal cord and from cranial nerves leading directly to it from the eyes, inner ear, etc. It then organizes these impulses. This organization process is the key to brain function: conscious sensation, memory, the association of one stimulus with another or with a memory, and the coordinated body action necessary for proper responsiveness all depend upon the circuits taken by nerve impulses within the brain. Furthermore, the initiation of impulses to be sent to the motor neurons of the body does not necessarily depend upon sensory impulses reaching the brain. It is quite clear that our brain can initiate body responses simply as a result of its own self-contained activity. An example of this would be action taken as a result of something suddenly remembered. The evidence suggests that man's brain is almost unique in this respect. The earthworm, grasshopper, and frog, for example, seem far more dependent on specific stimuli for initiating responses. We say that these creatures are more sense dominated.

The human brain consists of two large hemispheres (Fig. 27.7). Because of the crossing over of the spinal tracts, the left hemisphere of the brain controls the right side of the body and vice versa. The brain, as well as the spinal cord, is covered with three protective membranes, the **meninges** (Fig. 27.8). Cerebrospinal fluid is found between the inner two membranes and probably helps to cushion the brain from blows to the skull. Within the brain are four chambers, the ventricles, also filled with

Cerebrospinal fluid

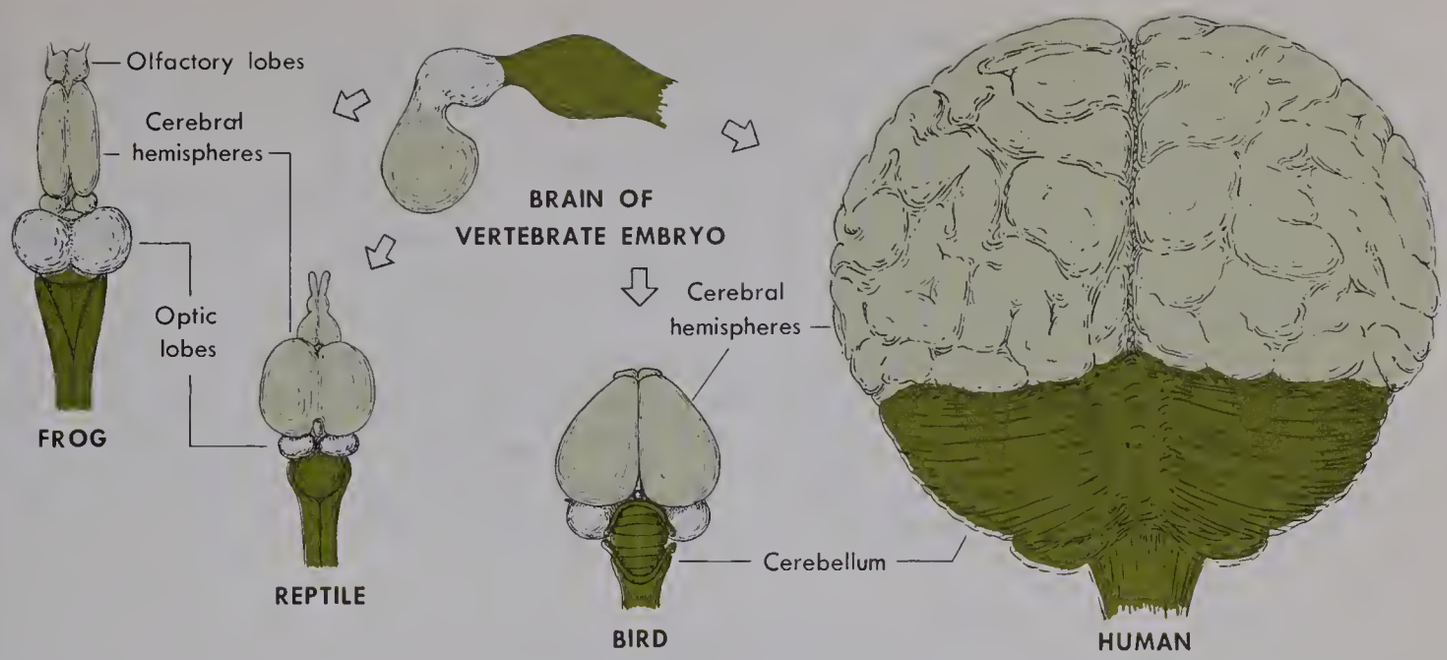


Fig. 27.7 The developing brain of the vertebrate embryo consists of three lobes. From these arise the structures of the forebrain (light color), midbrain (gray), and hindbrain (dark color). The human brain is shown from behind so that the cerebellum can be seen.

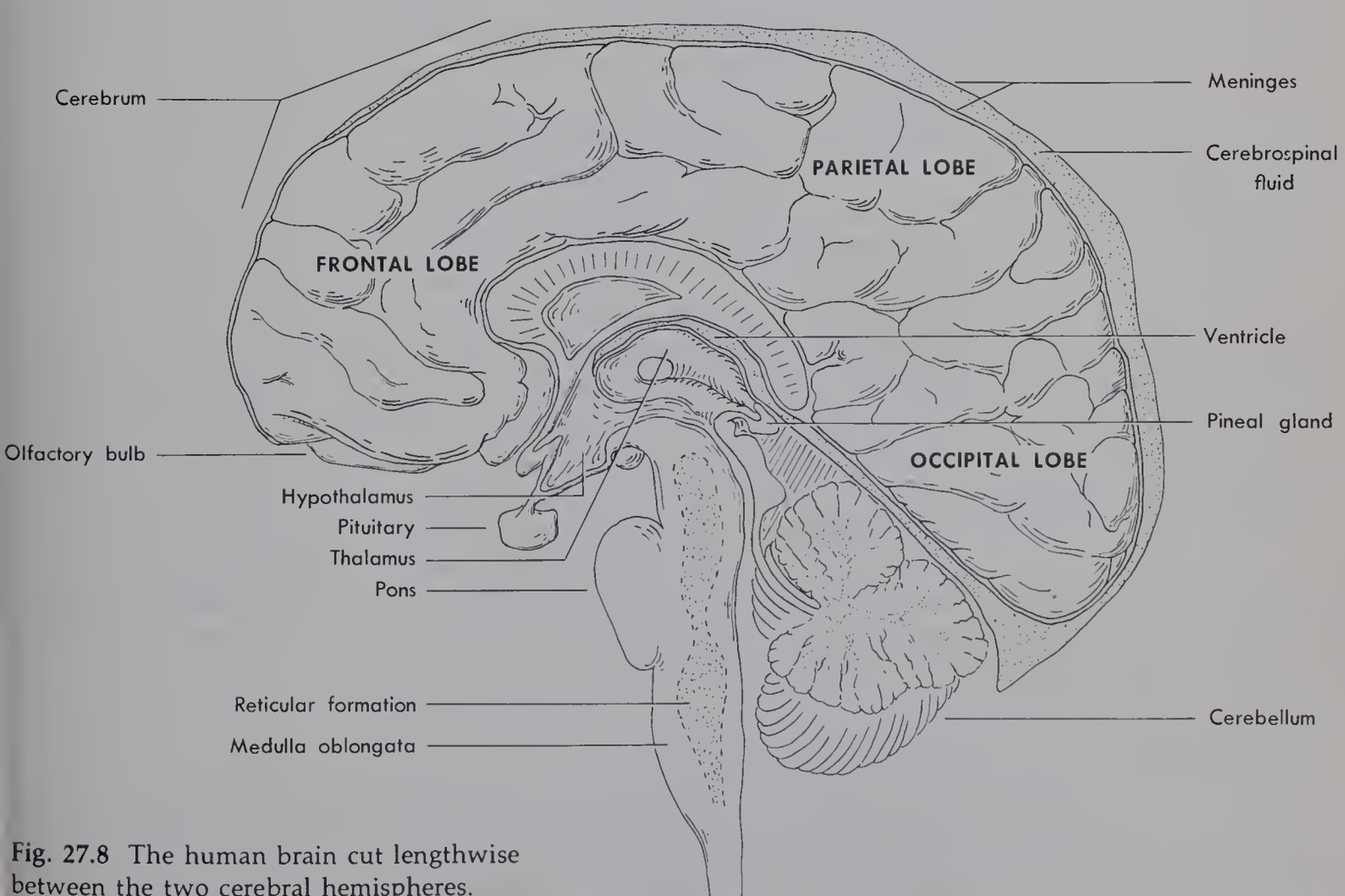


Fig. 27.8 The human brain cut lengthwise between the two cerebral hemispheres.

cerebrospinal fluid. There are large capillary beds within two of these ventricles, permitting the exchange of materials between the blood and the cerebrospinal fluid. The cells lining the ventricles are ciliated and keep the cerebrospinal fluid circulating.

Cranial nerves

Extending from the brain are twelve pairs of cranial nerves. Not all of these are mixed nerves; the optic nerves and olfactory nerves, for example, contain sensory neurons only.

The brain is divided into three regions: forebrain, midbrain and hindbrain. These divisions are not immediately obvious in the adult human brain because each is, in itself, made up of several parts or lobes. However, the pattern is clearly visible during the development of the brain in the embryo. The brains of all vertebrates are constructed on this basic plan (Fig. 27.7).

27.7 THE HINDBRAIN

The medulla oblongata

The two main portions of the hindbrain are the **medulla oblongata** and the **cerebellum.** The medulla oblongata has the appearance of simply a swollen tip of the spinal cord. Though small in size, it is absolutely essential to life. The nerve impulses which stimulate the intercostal muscles and diaphragm, and thus permit breathing, originate in the medulla. Nerves regulating heartbeat, diameter of the arterioles and other important functions also arise here. It is not surprising that destruction of the medulla brings about immediate death. We have no direct conscious control over the functions of the medulla although we can modify its action somewhat through the use of other brain centers.

The cerebellum

The cerebellum consists of two deeply convoluted hemispheres. Its most important function seems to be coordinating muscular activity in the body. Such activity is initiated by impulses arising in the motor area of the forebrain. These impulses not only travel down the spinal cord to the motor neurons but also pass into the cerebellum. As the body action is carried out, sensory impulses from the proprioceptors, the eyes, the semicircular canals, etc., are also sent to the cerebellum. The cerebellum then "compares" the information on what the body is actually doing to what the forebrain had instructed it to do. If a discrepancy exists, the cerebellum sends modifying signals to the forebrain so that the appropriate corrective signals can be sent out to the muscles. It is not surprising that birds have relatively large cerebella (Fig. 27.7) when we consider that they must be capable of moving swiftly and accurately in three dimensions of space while we and other earthbound animals spend most of our lives moving about on fairly flat surfaces.

27.8 THE MIDBRAIN

The midbrain in man is quite small and inconspicuous. It relays nerve impulses between the forebrain and hindbrain and between the forebrain and the eyes. It also participates in the maintenance of balance. Some other

vertebrates have relatively large midbrains. The prominent optic lobes of fishes, frogs, reptiles, and birds are part of their midbrains (Fig. 27.7).

The reticular formation

Running up through the center of the medulla oblongata and the midbrain is a network of nerve fibers known as the **reticular formation.** It serves to activate or awaken the forebrain. Sensory tracts of the spinal cord lead both to the forebrain and to the reticular formation, but the forebrain cannot respond to impulses reaching it from the sensory tracts unless it is first awakened by the reticular formation. As you might guess from your own experience, the reticular formation is quite selective in its action. It may not arouse the forebrain when large, but familiar, stimuli (such as traffic sounds) are received. A creaking floorboard, on the other hand, may produce instant wakefulness. Destruction of the reticular formation results in a permanent coma followed eventually by death.

27.9 THE FOREBRAIN

Parts of the forebrain

The most prominent part of man's forebrain is the **cerebrum.** It is made up of two large, deeply convoluted hemispheres. Each of these is subdivided into four lobes: frontal, parietal, occipital, and temporal (Fig. 27.8). In most other vertebrates (e.g. the frog) there are large olfactory lobes which are present as outgrowths of the cerebrum (Fig. 27.7), but this portion of the brain is relatively small in man. The forebrain also includes the thalamus, hypothalamus, part of the pituitary gland, and the pineal gland.

Surely no other body structure sets man so far apart from the other vertebrates as his cerebrum. The volume of the two cerebral hemispheres averages approximately 1350 ml. While a few large mammals such as whales have still larger cerebrums, the ratio of the size of the cerebrum to that of the rest of the central nervous system is far greater in man than in any other vertebrate.

Man and the other mammals have still another important feature in the organization of the cerebrum. The exterior of the cerebrum, the cortex,

Gray matter covers the forebrain of mammals

is made up of gray matter, masses of cell bodies. The myelinated nerve fibers of which white matter is composed are located *within* the cerebral hemispheres. This, you will recall, is just the reverse of the arrangement in the spinal cord. It is also the reverse of the arrangement in other vertebrate brains. The surface of the frog's cerebrum is glistening and white like all the rest of the central nervous system.

The significance of this reversal of pattern probably lies in the greater surface area it provides for the cell bodies. Many biologists feel that the extraordinary properties of our brain are dependent upon the enormous (over five billion) number of cell bodies present in the cerebral cortex and the unimaginably large number of possible connections which can be made between them. The many deep convolutions of the cerebral cortex provide additional surface area for these cell bodies to occupy.

Less than 1% of the neurons in the cerebrum send nerve fibers out of the cerebrum to other parts of the brain. What then are these vast numbers

of self-contained neurons accomplishing? To date, we can do little more than speculate. We do know that a great deal of electrical activity goes on in the cerebrum. Through use of the electroencephalograph, an instrument that detects and records brain "waves," we know that this electrical activity changes during sleep, wakefulness, excitement, etc. The instrument has even been used successfully to diagnose disorders of the brain such as a tendency toward epileptic seizures. Despite the limited success of this technique, we still know practically nothing of the internal activity of the cerebrum.

Mapping the cerebrum

Although the detailed electrical activity of the cerebrum is only dimly understood, some general functions of the cerebrum have been discovered. These discoveries have been made as a result of two kinds of studies. One is simply to destroy a portion of the cerebrum and see what happens to the victim. While this has been used with success on laboratory animals (using all the surgical precautions a human patient would receive), it is obviously risky to apply such findings to man. However, many cases of brain damage as a result of injury or infection have been studied in man and related to the presence of specific symptoms. The second technique is to expose the brain and then stimulate tiny portions of it with electrodes. Although this is of only limited use in laboratory animals, many humans undergoing brain surgery have volunteered to let such experiments be carried out on them while their brains were exposed. No pain is involved and when not under general anesthesia, the patients can report their sensations to the experimenter. Experiments of this sort have given us great insight into the function of the cerebrum. For example, they have revealed the presence of a band of cortex running parallel to and just in front of the fissure of Rolando (Fig. 27.9), which controls the action of the body's skeletal muscles. Stimulation of discrete areas within this band results in the contraction of the muscles controlled by that area. The larger the area of cortex involved, the more abundant the supply of motor neurons to the part of the body controlled by it.

A similar region has been discovered in a parallel band of cortex posterior to the fissure of Rolando. This region is concerned with *sensations* from the various parts of the body. When isolated spots are stimulated electrically, the patient reports sensation in some specific area of the body. A map can be prepared in accordance with such reports (Fig. 27.9).

The visual cortex

When portions of the occipital lobe are stimulated electrically, the patient reports the sensation of light. Not only does this region seem to be necessary for the primary act of seeing, but certain regions within it are necessary for associations to be made with what is seen. Damage to these regions may result in the person's being perfectly capable of seeing objects but incapable of associating them with previous experiences—of recognizing them. Such a defect is known as aphasia. The centers of hearing and understanding what is heard are located in the temporal lobes.

Although a few other functions have been assigned to specific areas of the cortex, the function of large areas of the cortex still remains unknown. Manipulation of these "silent" areas fails to reveal any specific

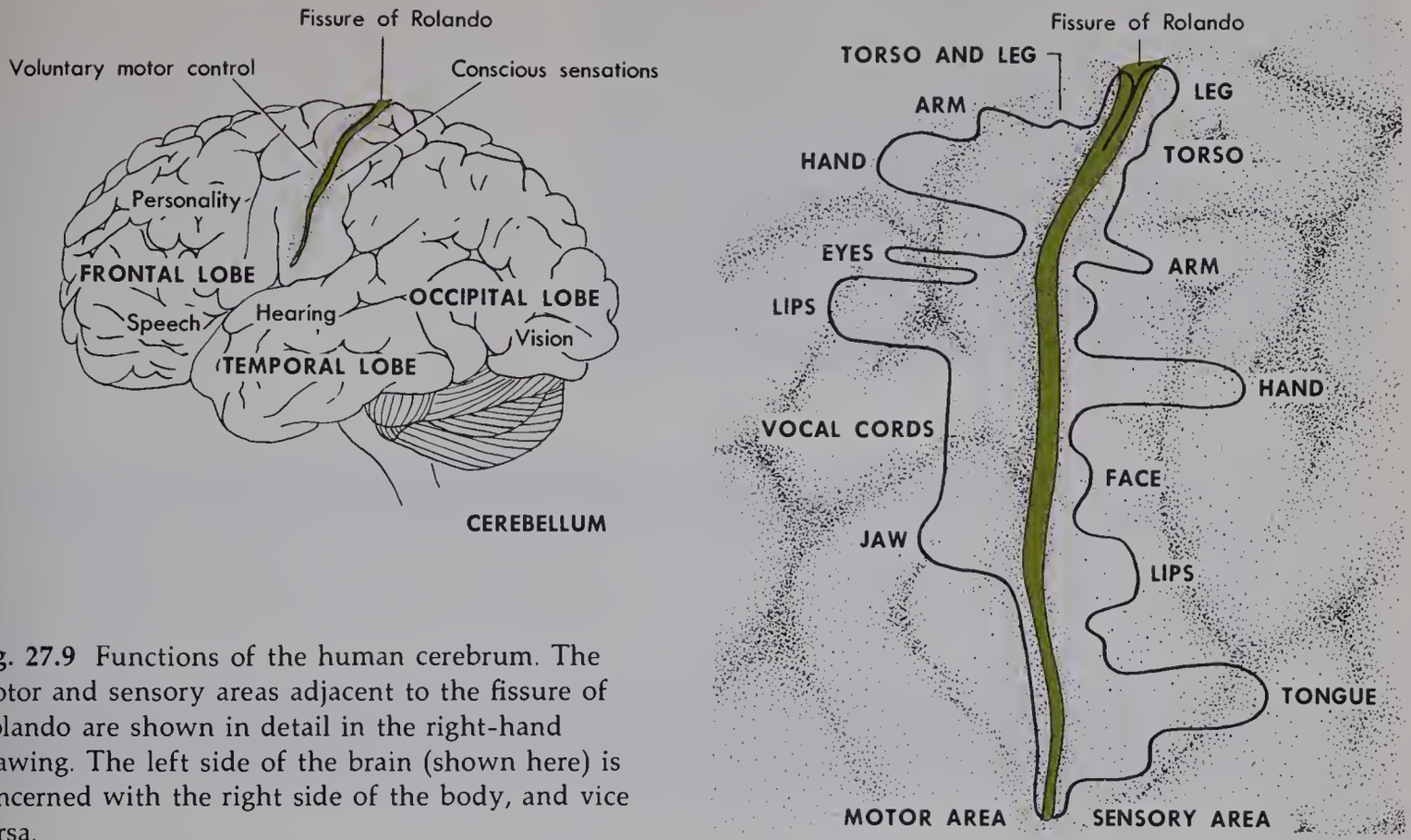


Fig. 27.9 Functions of the human cerebrum. The motor and sensory areas adjacent to the fissure of Rolando are shown in detail in the right-hand drawing. The left side of the brain (shown here) is concerned with the right side of the body, and vice versa.

activity. This is especially true of the large frontal lobes. Perhaps these large areas are responsible for carrying out some of man's "higher" mental activities. Learning, memory, logical analysis, foresight, creativity, and some emotions may all depend upon the nervous activity of the frontal lobes and other silent areas. The dependency must be quite general, though, because no one has yet succeeded in relating any one of these mental activities with any specific spot in the cortex. Perhaps the vast number of neurons involved enables one portion of these silent areas to take over the function of some other portion that has become damaged.

The story of Phineas P. Gage

Damage to the frontal lobes may produce changes in human behavior. Evidence of this was acquired in 1848 when a Vermont quarry worker, Phineas P. Gage, accidentally exploded some blasting powder by tamping it with a metal bar. The explosion drove the bar right through the front portion of his head, severely damaging his frontal lobes. Miraculously, he survived the accident. Furthermore, he lost none of the clearly defined functions of the brain. His vision, hearing, other sensation, speech, and body coordination were unimpaired. Nevertheless, a marked change in personality was soon noted. Formerly a reasonable, sober, conscientious person, Phineas became thoughtless, irresponsible, fitful, obstinate, and profane. In short, certain special, hard-to-measure, human qualities had been radically altered by his accident.

In 1935, 74 years after Phineas died (his skull now resides in the museum of the Harvard Medical School—with the bar resting nearby—





Fig. 27.10 Top: skull of Phineas P. Gage showing where a tamping bar entered (left) and exited (right) in an accident that occurred 12 years before he died of natural causes in 1861. Bottom: the tamping bar. (Courtesy of the Warren Anatomical Museum, Harvard University Medical School.)

Fig. 27.10), it was learned that destruction of the frontal lobes alleviates certain forms of mental illness. Presumably these mental derangements were brought on by excessive worry, feelings of guilt, etc., and were thus helped by destruction of the frontal lobes. In the operation, called prefrontal lobotomy, the lobes are not actually destroyed. Instead, the fibers connecting the frontal lobes to the thalamus are simply severed.

Prefrontal lobotomy

Today, the operation is performed only infrequently. For one thing, many desirable, human, attributes are irrevocably lost as a result of the operation. Furthermore, the development of tranquilizing drugs has permitted doctors to achieve the same type of improvement in a much less drastic way.

Our discussion of the forebrain would not be complete without mention of the thalamus and hypothalamus (Fig. 27.8). The thalamus is the "gatekeeper" of the cerebral cortex. All sensory messages reaching the brain must pass through the thalamus in order to be sensed consciously.

The hypothalamus

We have already discussed some of the important functions carried out by the hypothalamus. In addition to monitoring and regulating the temperature and water content of the blood, the hypothalamus is the coordinating center for many of the activities of our internal organs. In other animals, and perhaps in man, the hypothalamus is the center of such "feelings" as thirst, hunger, satiety, sex drive, and rage. The hypothalamus not only has nervous activity but also, as we have seen, produces hormones. Two of these (oxytocin and vasopressin) are stored in the posterior lobe of the pituitary before being released into the bloodstream. Others pass to the anterior lobe of the pituitary in veins draining the hypo-

thalamus. There they stimulate the release of the anterior lobe's own hormones.

27.10 THE PROCESSING OF VISUAL INFORMATION

In most respects, the human brain remains a "black box." We know something about what goes in and what comes out, but very little of what actually goes on *in* it. We know that the brain is constructed of many interconnecting neurons. We know that neurons can either "fire" or not. These features cannot help but remind us of the organization of a computer: myriads of interconnecting circuits that are, at any moment, either "on" or "off." But what exactly does the brain do with sensory impulses reaching it? How does the brain "process" information?

The question remains to a large extent unanswered, but at least a solid start has been made in determining how the brain processes information reaching it from the eyes. In fact, the processing starts within the eyes. (This should not be surprising inasmuch as the retina is actually an extension of the brain.)

The retina

The basic strategy is to use a tiny electrode like those described earlier in the chapter to look for impulses in the neurons of interest. Work with a variety of animals has demonstrated that any single visual receptor will fire if light of sufficient intensity falls upon it. **Rods** and **cones** synapse in the retina with **bipolar cells** which, in turn, synapse with **ganglion cells** (Fig. 26.7). It is the axons of the ganglion cells that make up the optic nerve. As we learned in the last chapter, each receptor cell does not enjoy its own private circuit back to the brain. There are some 10^8 rods and cones in one human eye but only some 10^6 ganglion cell axons that make up the optic nerve. Thus a given ganglion cell must receive inputs from a number of receptor cells.

Examination of the retina shows that a single bipolar cell receives input from several receptor cells and, similarly, a single ganglion cell receives inputs from several bipolar cells.

By inserting a delicate recording electrode into a single ganglion cell and then shining light on the retina, Stephen W. Kuffler demonstrated what goes on in the early steps of visual processing. Even in the dark, ganglion cells have a slow, steady rate of firing. Diffuse light directed on the retina has little effect on this rate. A tiny spot of light, however, can either increase *or decrease* the rate of firing. A given ganglion cell either (1) increases its activity when light falls on a small circular area of the retina and *decreases* it when the light falls on an area of the retina concentric with the first area (Fig. 27.11), or (2) does just the reverse. We assume that light shining upon "off" areas triggers bipolar cells that release an *inhibitory* transmitter at the ganglion cells stimulated by the "on" areas. Diffuse light which illuminates both "on" and "off" areas causes them to cancel each other. Thus the optic nerve is not telling the brain that light has been detected but that contrasts between light and dark (i.e. shapes) have been detected.

"On"-center receptive fields

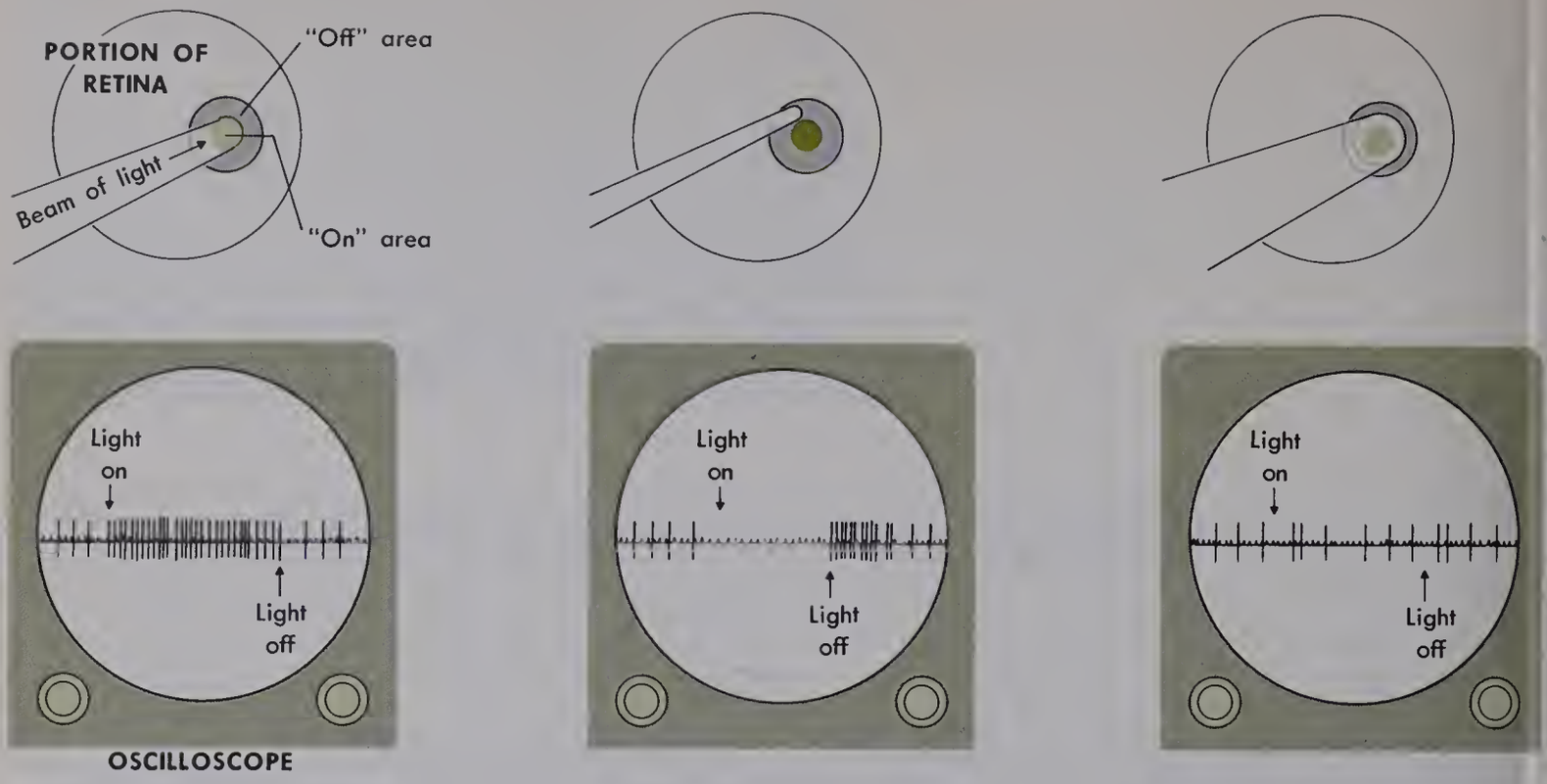


Fig. 27.11 Response of a ganglion cell to illumination of the retina. Left: light falling on a small circular area of the retina increases the activity of the ganglion cell. Center: light directed around the perimeter of the "on" area suppresses that ganglion cell. Right: light shining on both areas produces no effect. Other ganglion cells have a central "off" area surrounded by an "on" area.

The lateral geniculate body

The axons of the optic nerve pass back close to the center of the base of the forebrain to the so-called **lateral geniculate body** (Fig. 27.12), where they synapse with a new set of association neurons. The axons of these cells lead up and back to the visual cortex. Two associates of Kuffler, David H. Hubel and Torsten N. Wiesel, have pursued the processing of visual information in these areas. Again they insert microelectrodes in the areas of interest, but instead of directing light into the eye, they project images on a screen placed in front of the animal (a cat or a monkey). The animal is anesthetized and the eye prevented from moving. Using this procedure, Hubel and Wiesel found that the cells of the lateral geniculate body respond about the same way that the ganglion cells do, but even more so.

The visual cortex

The story was quite different in the visual cortex. The first cells in the visual cortex, the so-called "simple cells," no longer respond to circles of light but only to bars of light (or dark) or to straight-line edges between dark and light areas (Fig. 27.12). Only when the stimulus is directed at one area of the screen, and only at a given angle, will a single simple cortical cell respond. (Of course, the ineffective positions for one cortical cell result in stimulation of *other* cortical cells.)

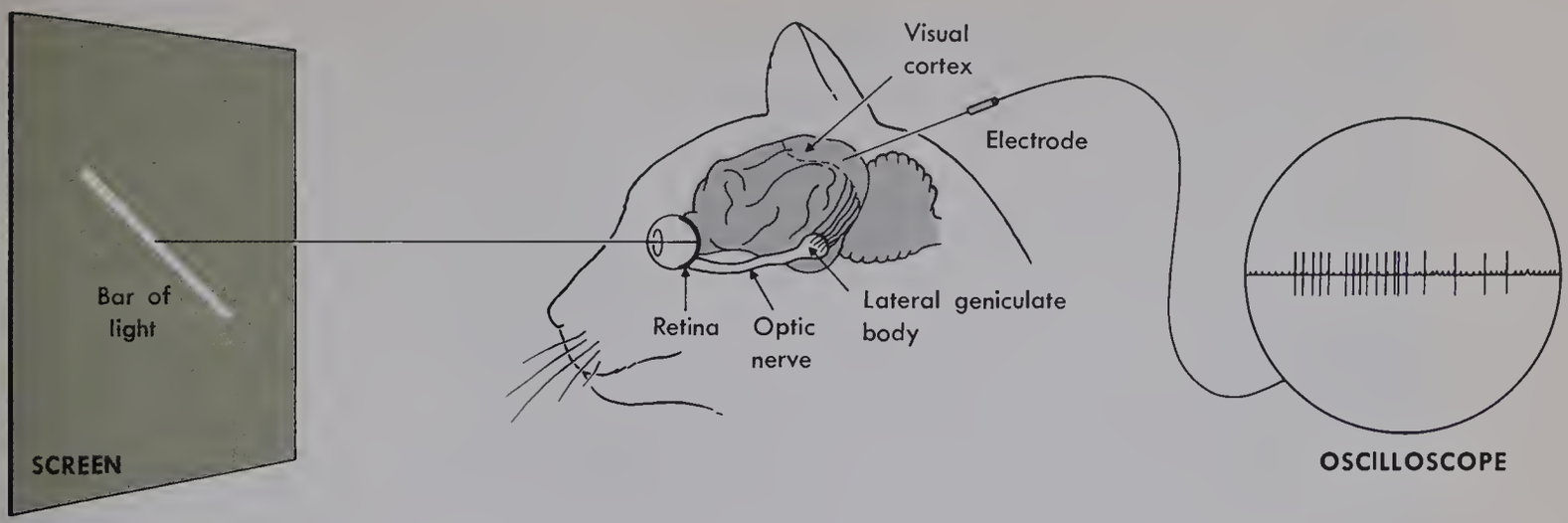


Fig. 27.12 Experimental procedure for monitoring the response of neurons in the visual cortex to various visual stimuli. In a similar way, the activity of ganglion cells (in the optic nerve) and of neurons in the lateral geniculate body can be studied by placing the electrode in those areas.

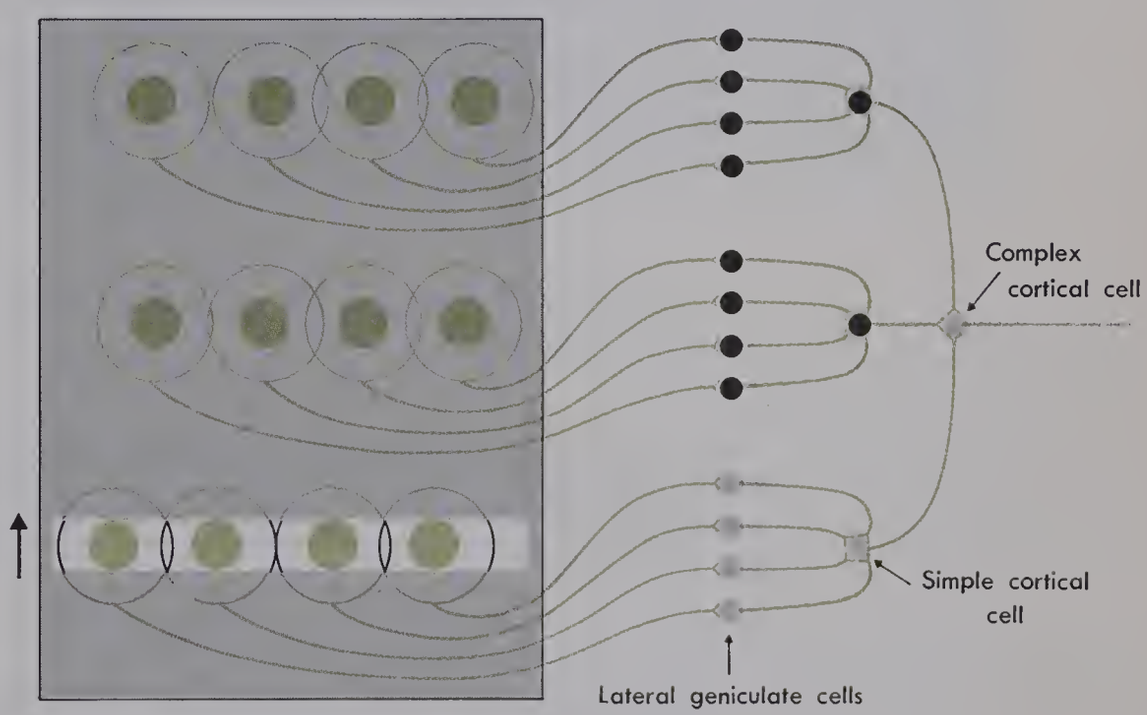


Fig. 27.13 Mechanism by which the circular response areas of ganglion and lateral geniculate body cells may be converted into the rectangular response areas characteristic of simple cortical cells. Movement of the bar of light in the direction shown would continue to activate the complex cortical cell.

The preference that simple cortical cells have for lines can be explained if we assume that these cells can be activated only if they receive inputs from a number of lateral geniculate cells whose areas (circular) of response are arranged in a line (Fig. 27.13).

Complex and hypercomplex cells

Probing elsewhere in the visual cortex, Hubel and Wiesel have demonstrated the existence of still other association neurons ("complex" and "hypercomplex") that carry out still more processing. Complex cells still want their edges oriented in one direction, but they can now be moved over a substantial area of the screen. This makes sense if a series of simple cortical cells—all responding to an edge of the same slope, but each respon-

sible for a different part of the visual field—converge on a single complex cell. Thus a complex cell continues to respond to a given stimulus even though its absolute position on the retina changes (Fig. 27.13).

The "hypercomplex" cells respond best when the straight-line stimulus, still with a definite slope, is limited in length at one or both ends. Right-angle corners often serve as powerful stimuli for these cells.

"Processing" involves the selective destruction of information

The story of how the brain processes visual information is obviously not complete. But even so, we can now get a glimpse of the essential job that association neurons in the brain carry out. At each level of processing (bipolar cells to ganglion cells, lateral geniculate cells to simple cortical cells, etc.), the inputs of several association neurons are funneled into a single output. Thus at each step some of the visual information is selectively destroyed. Simple cortical cells, for example, will fire only if a number of lateral geniculate cells converging on them are simultaneously active (see Section 27.4). If not, the excitation dies out at the synapses. In this way, each level of the brain serves as a filtering device and, in so doing, provides the mechanism by which certain features of what might be a very complex stimulus can be discriminated. This pioneering work on visual processing suggests that the mammalian brain responds not to particular impulses generated in particular circuits, but to the spatial and temporal organization of many impulses passing along a multitude of converging circuits.

THE PERIPHERAL NERVOUS SYSTEM OF MAN

The peripheral nervous system is made up of the sensory and motor nerve fibers which run to and from the central nervous system and the rest of the body. It can be subdivided into the sensory-somatic system and the autonomic system.

27.11 THE SENSORY-SOMATIC SYSTEM

Cranial and spinal nerves

The sensory-somatic system consists of 12 pairs of cranial nerves, not all of which are *mixed* nerves (see Section 27.4), and 31 pairs of spinal nerves, all of which are mixed. These nerves transmit impulses from our receptors (chiefly of external stimuli) to the central nervous system. They also transmit impulses from the central nervous system to all the skeletal muscles of the body.

Impulses traveling down motor neurons of the sensory-somatic system cause the skeletal muscle fibers at which they terminate to contract. Body movement results. The junction between the terminal end of the motor neuron and the muscle fiber is called the **neuromuscular junction.** Its properties are quite like those of a synapse. The tips of the motor axons

The neuromuscular junction is a type of synapse

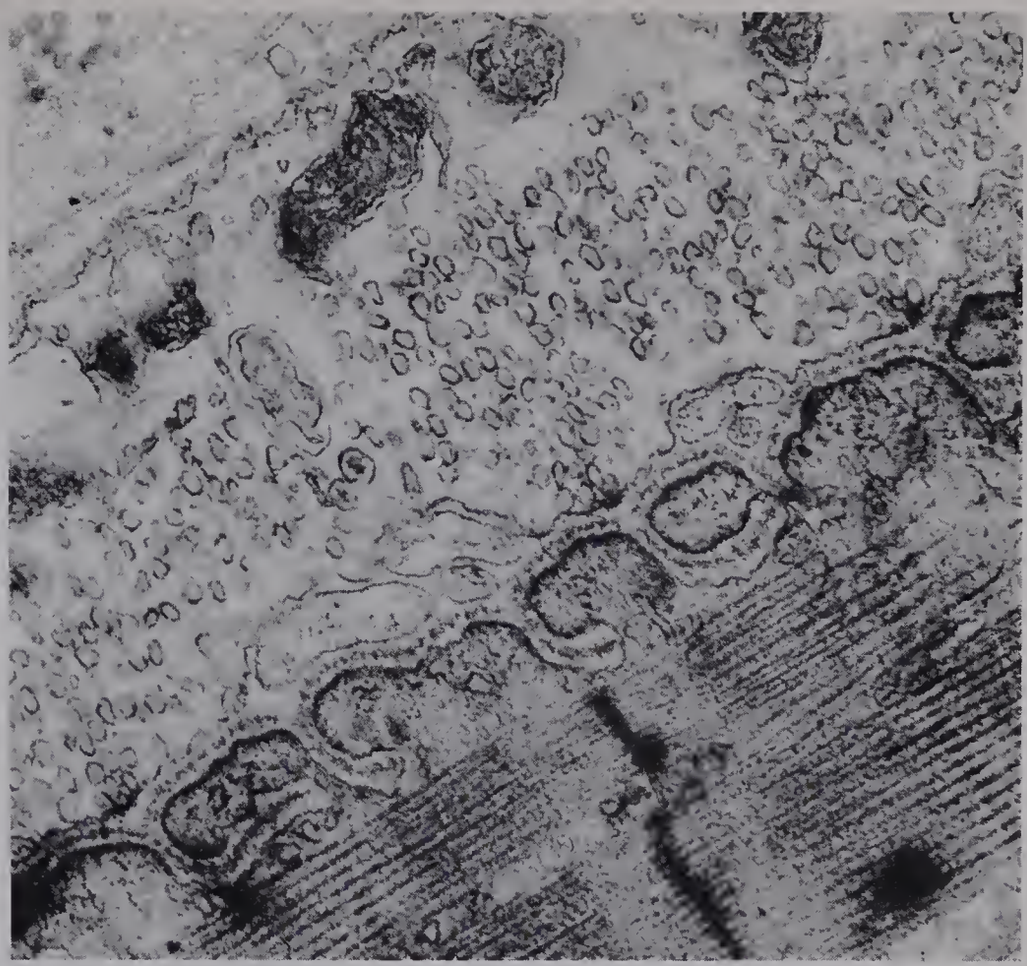


Fig. 27.14 The neuromuscular junction. Many vesicles can be seen in the portion of the motor end plate shown. These are thought to contain acetylcholine. Its release into the gap next to the folded membrane of the muscle fiber (running diagonally upward from the lower left) initiates the events leading to contraction. (Courtesy of Prof. B. Katz.)

are called motor end plates (Fig. 27.14). They contain thousands of tiny vesicles, in or on which are molecules of ACh. When a nerve impulse reaches the motor end plate, hundreds of vesicles release their ACh onto the surface of the muscle fiber. As is the case in a synapse, this reduces the membrane potential of the muscle fiber and initiates a stimulating impulse along it. Muscle contraction follows. With the job completed, acetylcholinesterase destroys the liberated ACh and leaves the field cleared for another impulse.

All our conscious awareness of the external environment and all our motor activity to cope with it operate through the sensory-somatic portion of the peripheral nervous system (Fig. 27.15).

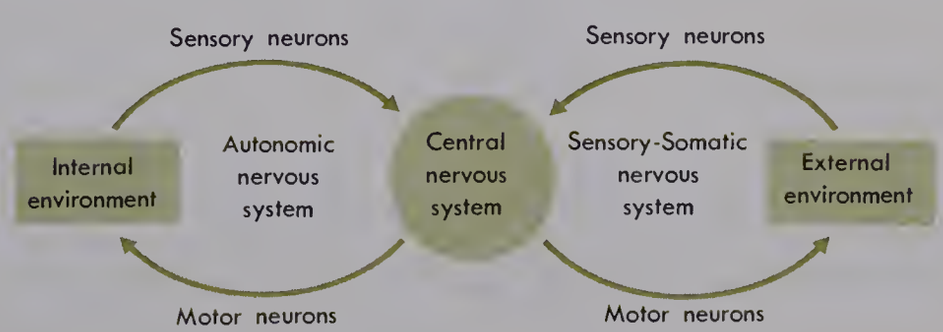


Fig. 27.15 Relationship between the three major divisions of the nervous system.

27.12 THE AUTONOMIC NERVOUS SYSTEM

The autonomic nervous system consists of sensory and motor neurons running between the central nervous system (especially the hypothalamus) and the various internal organs: the heart, viscera, and many glands, both exocrine and endocrine. It is thus responsible for detecting certain conditions in the internal environment and bringing about appropriate changes in them.

Preganglionic and postganglionic autonomic motor neurons

The actions of the autonomic nervous system are largely involuntary in contrast to those of the sensory-somatic system. Another difference between the two systems is that two groups of motor neurons are used to stimulate the effectors instead of just one. The first, or *preganglionic,* neurons arise in the central nervous system and run to a ganglion in the body. Here they synapse with the second, *postganglionic,* neurons which run to the effector. The autonomic nervous system has two subdivisions, the sympathetic and parasympathetic nervous systems, each with its special organization and functions.

27.13 THE SYMPATHETIC NERVOUS SYSTEM

Sympathetic ganglia

The preganglionic motor neurons of the sympathetic nervous system arise in the spinal cord. They leave by way of the ventral root of a spinal nerve and pass into a sympathetic ganglion (Fig. 27.16). These ganglia are organized into two chains running parallel to and on either side of the spinal cord (Fig. 27.17). The preganglionic neuron may do one of three things in the sympathetic ganglion. It may (1) synapse with postganglionic neurons which then re-enter the spinal nerve and ultimately pass out to the sweat glands and the walls of blood vessels near the surface of the body, (2) pass up or down the sympathetic chain and finally synapse with postganglionic

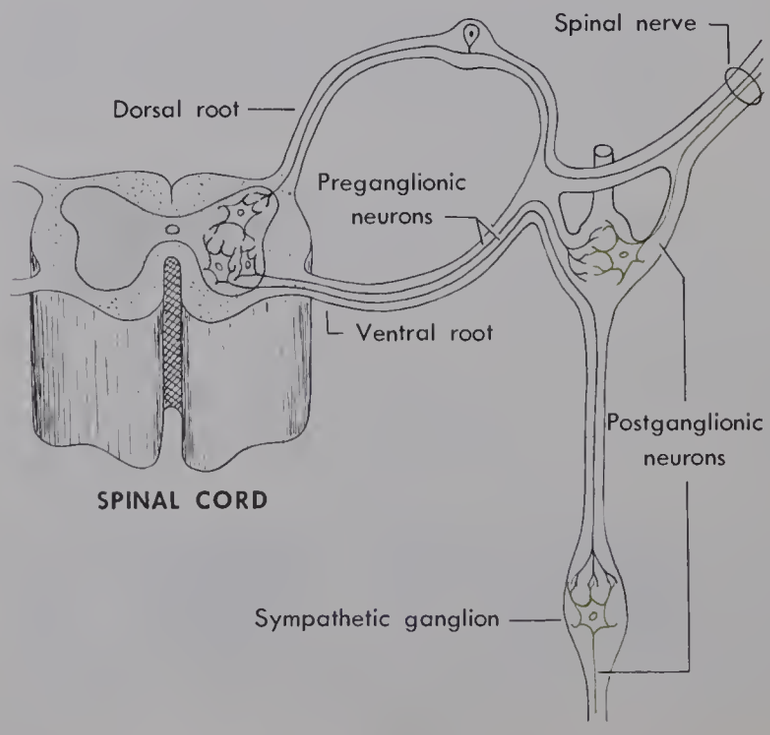


Fig. 27.16 Pathways of the sympathetic neurons. The preganglionic neurons are shown in black; the postganglionic neurons, in color.

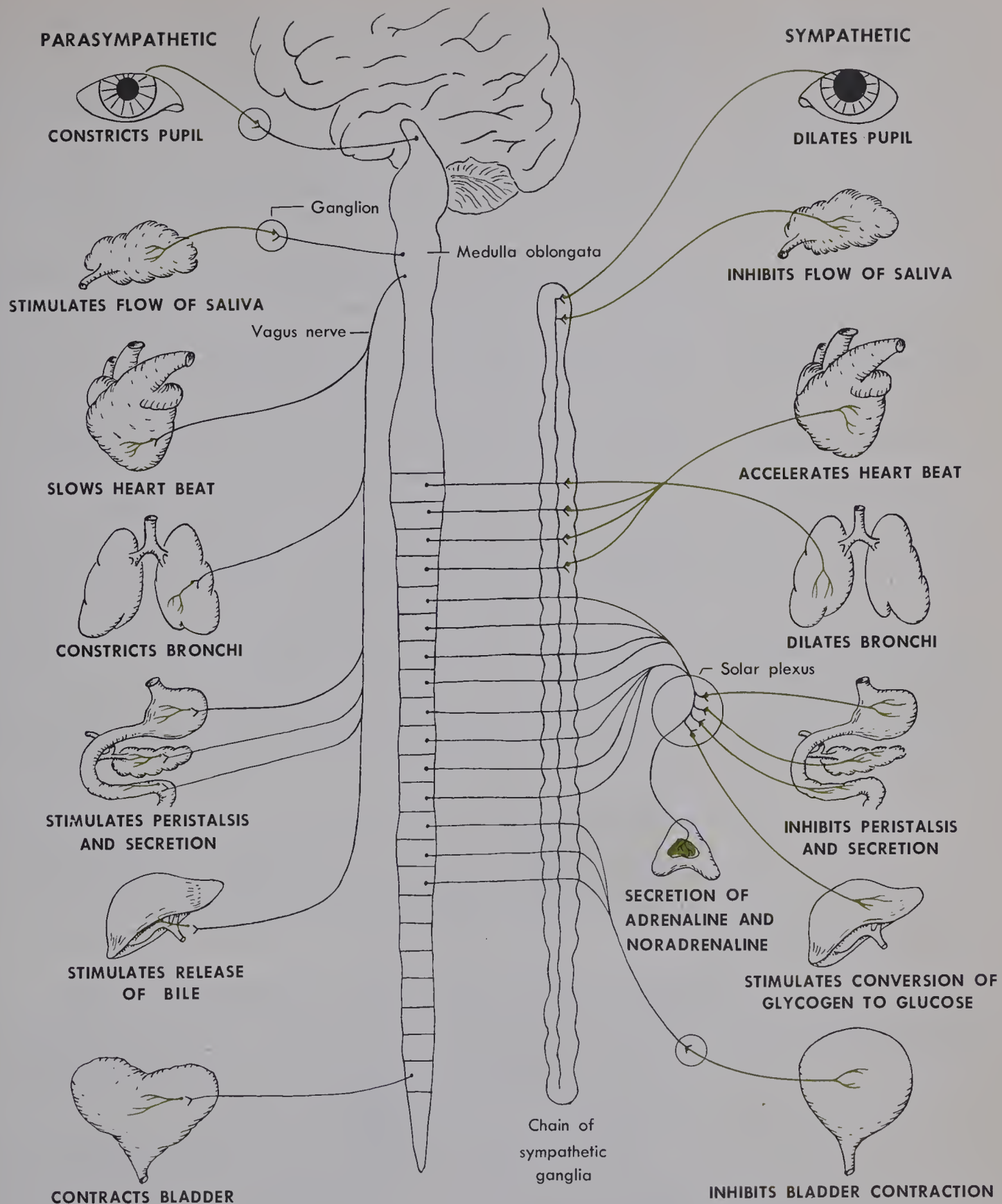


Fig. 27.17 The autonomic nervous system. The sympathetic nerves prepare the body for emergencies. The parasympathetic nerves reverse the effects of sympathetic stimulation. The preganglionic neurons are shown in black; the postganglionic neurons, in color.

neurons in a higher or lower ganglion, or (3) leave the ganglion by way of a cord leading to special ganglia (e.g. the solar plexus) in the viscera (Fig. 27.17). *Here* it may synapse with postganglionic neurons running to the muscular walls of the viscera. However, some of these preganglionic sympathetic neurons pass right on through this second ganglion and into the adrenal medulla. Here they synapse with the highly modified postganglionic cells which make up the secretory portion of the adrenal medulla.

The transmitter substance of the preganglionic sympathetic neurons is ACh. It serves to transmit impulses to the postganglionic neurons. A chemical stimulator is also released by the end plates of the postganglionic neurons. In most cases, this is noradrenaline, although in some other animals (for example the frog) the sympathetic fibers release adrenaline instead. The action of noradrenaline (or adrenaline) on a specific gland or muscle is excitatory in some cases, inhibitory in others. Its release by these end plates stimulates heartbeat, raises blood pressure, dilates the pupils, dilates the trachea and bronchi, and stimulates the conversion of liver glycogen into glucose. Sympathetic stimulation also shunts blood away from the skin and viscera to the skeletal muscles, brain, and heart. It inhibits peristalsis in the alimentary canal and contraction of the bladder and rectum. In short, stimulation of the sympathetic branch of the autonomic nervous system duplicates most, if not all, of those actions carried out by the adrenaline and noradrenaline released into the blood by the adrenal medulla. This should not be surprising when we remember that the adrenal medulla really is a part of the sympathetic nervous system, its secretory cells being modified postganglionic cells.

Actions of noradrenaline

The adrenal medulla is part of the sympathetic nervous system

The actions produced by stimulation of the sympathetic nervous system are quite general. There are two reasons for this. One is that a single preganglionic neuron usually synapses with many postganglionic neurons. What starts as a single impulse becomes magnified. Second, the release of adrenaline and noradrenaline into the bloodstream ensures that every cell of the body will be exposed to these substances when necessary, even if no postganglionic neurons reach them directly.

27.14 THE PARASYMPATHETIC NERVOUS SYSTEM

The vagus nerves

The main nerves of the parasympathetic system are the tenth cranial nerves, the **vagus** nerves, which arise in the medulla oblongata. Other preganglionic parasympathetic neurons also extend from the brain as well as the lower tip of the spinal cord (Fig. 27.17).

Each preganglionic parasympathetic neuron synapses with just a few postganglionic neurons, which are located near or in its effector organ, a muscle or gland. The synaptic knobs of the preganglionic neurons and the end plates of the postganglionic neurons both release acetylcholine. Stimulation of the parasympathetic nerves causes a slowing down of heartbeat, lowering of blood pressure, constriction of the pupils, increased blood flow to the skin and viscera, and promotes peristalsis in the alimentary canal. In brief, the parasympathetic nervous system serves to return our body

Actions of acetylcholine

functions to normal after they have been altered by sympathetic stimulation. In times of danger, the sympathetic nervous system prepares us for violent physical activity such as fighting or fleeing. These changes would be harmful if prolonged unnecessarily, and the parasympathetic system reverses them when the danger is over. We must therefore include the antagonistic activity of these two branches of the autonomic nervous system among the most important body mechanisms for maintaining homeostasis.

Loewi's experiments

The discovery that specific chemical substances are released when either branch of the autonomic nervous system is stimulated was made by the Nobel Prize-winning physiologist Otto Loewi in 1920. He carefully removed the living heart of a frog with its sympathetic (*accelerator*) and parasympathetic nerve supply intact. As was expected, electrical stimulation of the first speeded up the heart while stimulation of the second slowed it down.

Loewi also found that these two responses would occur in a second frog heart if he simply bathed it with Ringer's solution taken from the stimulated heart (Fig. 27.18). This showed that chemical substances were produced by the first heart which duplicated the action of nervous stimulation. In the case of parasympathetic (vagus) stimulation, the substance was later identified as acetylcholine. During sympathetic stimulation, adrenaline (in the frog) is released.

Meditation and the autonomic nervous system

While the autonomic nervous system is considered to be involuntary (as its name suggests), this is not entirely true. A certain amount of conscious control can be exerted over it, as has long been demonstrated by some practitioners of the Eastern religions of yoga and Zen Buddhism.

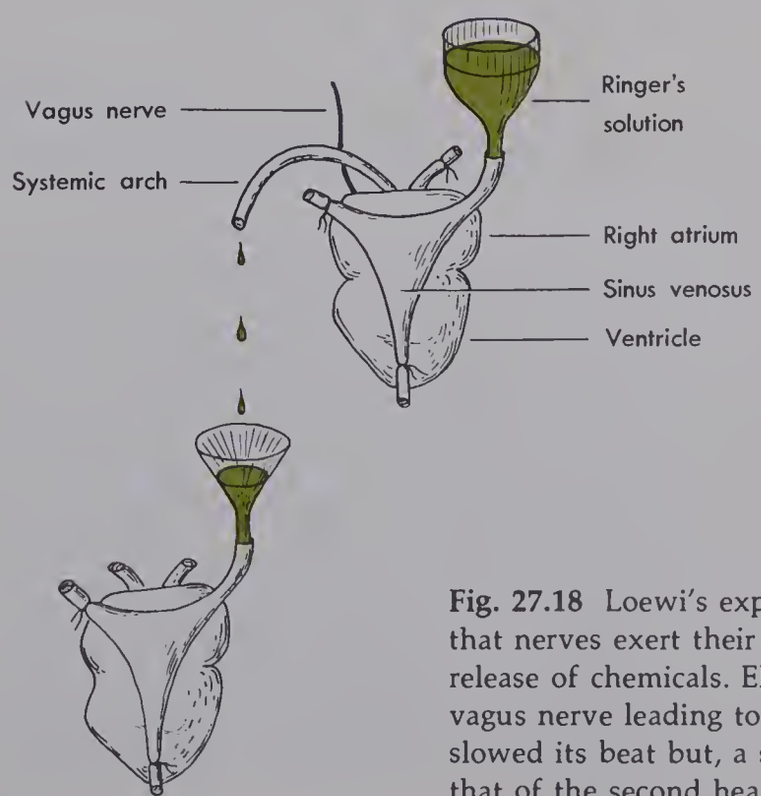


Fig. 27.18 Loewi's experiment, which showed that nerves exert their effects through the release of chemicals. Electrical stimulation of the vagus nerve leading to the first heart not only slowed its beat but, a short time later, slowed that of the second heart, too.

During their periods of meditation, these people are clearly able to alter a number of autonomic functions including rate of heartbeat and rate of oxygen consumption. These changes are not simply a reflection of decreased physical activity, because they greatly exceed the amount of change which occurs during sleep or hypnosis (Fig. 27.19). Neal Miller and his coworkers, working in the United States, have demonstrated that dogs and laboratory rats can be trained to alter, either by an increase or a decrease (as the experimenter desires), such accepted autonomic functions as blood pressure, rate of heartbeat, peristalsis, and distribution of blood to various capillary beds. As a consequence of these discoveries, attempts are now being made to train humans with high blood pressure or heart rate to lower these to normal levels. While such learning has been successfully acquired for a time, whether it can be retained for useful periods after the training program is stopped is yet to be demonstrated.

Visceral learning

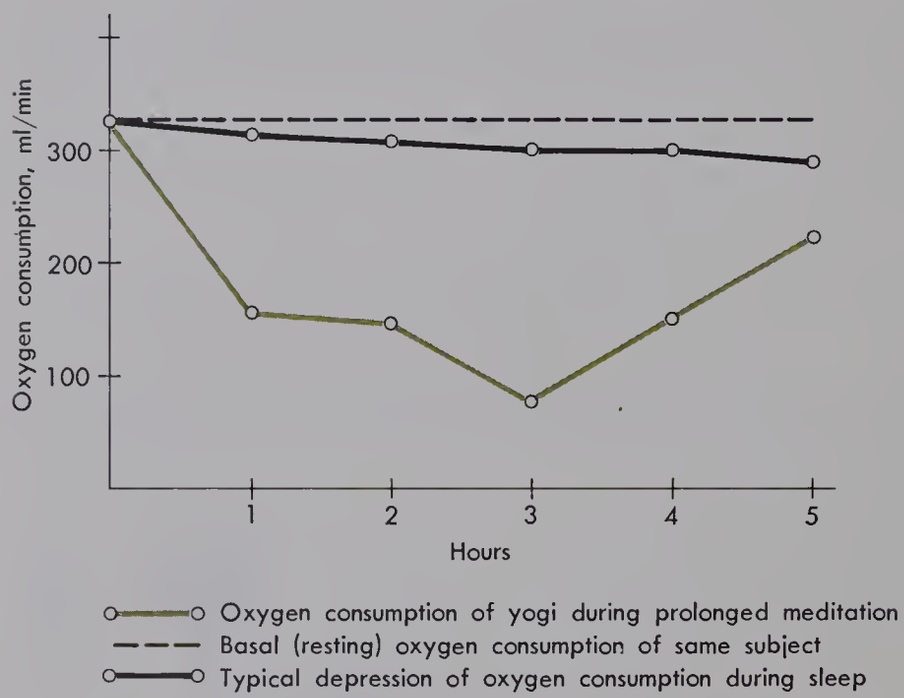


Fig. 27.19 Effect of meditation on oxygen consumption. Properly-trained individuals can consciously alter a number of other functions that are controlled by the autonomic nervous system.

27.15 DRUGS AND THE NERVOUS SYSTEM

The activity of the nervous system depends upon a well-orchestrated ensemble of biochemical activities. Some of these, e.g. energy release by cellular respiration, closely resemble those found elsewhere in the body. Others, such as the synthesis, release, and action of transmitter substances, are unique to the nervous system. In either case, there are many opportunities for altering nervous system activity by administering chemicals that mimic or block, or in some other way alter, one or more of its biochemical activities. In view of the manifold activities of the nervous system, it should be no surprise that alterations of its activity lead to alterations in behavior and consciousness. Perception, muscular coordination, and emotions are among the functions of the brain that can be greatly altered by chemicals.

Psychoactive drugs

There is plenty of evidence that man has deliberately consumed certain chemicals (e.g. ethyl alcohol, cocaine) since early in his history for the sake of achieving what he felt were beneficial or pleasurable alterations in feelings and behavior. In recent years, the success of organic chemistry in synthesizing an enormous variety of psychoactive chemicals or drugs has greatly enlarged the possibilities for altering nervous system activity. Much of this research has led to advances in helping the mentally ill. But, concurrently with these advances, there has grown up an increasing interest in and consumption of these substances among people with no history of mental illness. While the use of psychoactive drugs is found among all ages and all peoples, the recent increase in the variety of drugs used has been especially dramatic among young people in the developed countries of the world.

Research techniques

The growth of what is often called the drug culture has provided the impetus for additional research into the mode of action of these drugs. However, the knowledge gained is as yet fragmentary. Several approaches have been taken. One is to administer the drug, either to humans or to experimental animals, and then to attempt to correlate the physiological and behavioral changes observed with known brain functions. Another approach is to administer radioactively labeled drugs to an experimental animal and see if and where they become concentrated within the brain. A good deal of effort has also gone into analyzing isolated preparations, that is, the *in vitro* effect of a drug on enzyme systems, properties of cell membranes, etc.

In considering the extraordinary diversity of psychoactive drugs, it soon becomes apparent that they fall into three major categories with respect to their physiological and behavioral effects. These are the stimulants, the depressants, and the hallucinogens.

Stimulants

Stimulants activate the sympathetic nervous system

The most widely used stimulants are caffeine (in coffee, tea, and cola beverages), nicotine (in cigarettes), and the amphetamines. Each of these stimulates the sympathetic nervous system, presumably through controlling centers in the hypothalamus. Each of the activities (e.g. acceleration of heart rate, dilation of the pupils, increase in blood sugar) described in our discussion of the adrenal medulla (see Section 25.10) and of the sympathetic nervous system (see Fig. 27.17) is promoted by these drugs. The sympathetic stimulation brought about by caffeine is very mild; by nicotine somewhat less so; and by the amphetamines, e.g. dexedrine, methylamphetamine ("speed"), quite powerful. Because of the role of the adrenal medulla and the rest of the sympathetic system in preparing the body for stress, it is not surprising that many athletes have turned to amphetamines in an effort to improve their performance. The few studies that have been made indicate that some types of athletic performance (e.g. running) may be enhanced following use of amphetamines—perhaps mainly from a reduced sense of fatigue. Activities requiring complex in-

Amphetamines

teractions with teammates are not improved and, in fact, deteriorate following use of amphetamines.

Diet pills

The amphetamines also affect other functions associated with the hypothalamus, such as increasing thirst and decreasing hunger and sleepiness. Because of their depressant effect on appetite, amphetamines have been widely used to help people lose weight. What little long-term success they have brought about seems to be far outweighed by the psychological and physical deterioration that constant stimulation of the sympathetic nervous system produces.

Cocaine

Cocaine is also a central nervous system stimulant. It has been used for thousands of years by certain tribes in the Andes of South America. It has no good medical use, and its nonmedical use in other countries has not approached that of the other stimulants.

Depressants

As the name suggests, the depressants reduce nervous system activity. There are five major categories of depressants. These are:

1. Ethyl alcohol.
2. Barbiturates. These include such drugs as Seconal, Nembutal, and Amytal.
3. Tranquilizers. The most widely used tranquilizers are meprobamate (trade names: Miltown and Equanil), chlorpromazine (Thorazine), chlordiazepoxide (Librium), and diazepam (Valium).
4. Opiates. These include opium, morphine, heroin, codeine, and methadone.
5. Anesthetics. These include ether, chloroform, and a number of other volatile hydrocarbons that are used as solvents (e.g. benzene, toluene, and carbon tetrachloride).

Ethyl alcohol is, by a wide margin, the most widely used depressant drug, not only in the United States but in most of the world. While its precise mode of action is obscure, its actions suggest a general reduction in neuron function in the brain. It appears that the sensitivity of the brain to inhibition decreases from the "top" of the system to the "bottom," i.e. from the frontal lobes of the forebrain to the medulla oblongata of the hindbrain. Thus the first effects of alcohol intoxication occur in the frontal lobes. The resulting removal of inhibitions may well give rise to the illusion that the drug is actually stimulatory. As the concentration of alcohol in the blood increases, one can watch the progressive changes resulting from the depression of lower and lower brain centers. Loss of dexterity and insensitivity to touch occur as the motor and sensory regions of the cortex become inhibited. Depression of the visual, auditory, and speech areas of the cortex leads to distorted vision, interference with hearing, and difficulty in speaking. At still higher alcohol levels, coordination and balance

Differential sensitivity of the brain to ethyl alcohol

are lost, presumably reflecting inhibition of the cerebellum. Depression of the reticular formation produces unconsciousness and, later, coma. In rare cases, people have ingested enough alcohol to depress the medulla oblongata to the point where breathing stops and death occurs.

Barbiturates

The barbiturates mimic some of the actions of alcohol, particularly in their ability to depress the reticular formation (thus promoting sleep) and, in high doses, the medulla oblongata (thus producing respiratory failure). As one might expect, barbiturates and alcohol act synergistically, the combination producing a depression greater than either produces alone. The combination is a frequent cause of suicide, both accidental and planned. It is estimated that barbiturate-alcohol poisoning is responsible for two-thirds of the suicides in the United Kingdom.

There are several other depressant drugs that share most of the properties of the barbiturates but are not related to them chemically. Prominent among these is methaqualone, a widely used (and abused) sedative. Like barbiturates, methaqualone acts synergistically with alcohol, and the combination may so depress the medulla oblongata that breathing stops.

Tranquilizers

Tranquilizers constitute another group of depressants that have seen greatly expanded use in recent years. These drugs act like barbiturates in reducing anxiety and tensions, but they do not share their sleep-inducing effects. Some of the more potent tranquilizers have been of enormous medical benefit in the treatment of psychotic patients.

Opiates

Opium is a mixture of substances from which a number of other "opiates" can be derived. These substances seem to depress the thalamus —the gateway for sensory impulses reaching the cerebral cortex—which would explain their effectiveness as pain killers. Morphine and codeine are used in large amounts as part of legitimate medical practice. Heroin is still more effective as a pain killer but is so highly addictive that its use is illegal in the United States. Methadone is a synthetic opiate that is now receiving extensive trials as a means of breaking addiction to heroin.

Although the opiates (chiefly heroin) are perhaps the most dramatically addictive drugs, they share this property with *all* the depressants described so far. Each of these drugs produces two related physiological effects. One is tolerance, that is, the necessity for a steadily increasing dose in order to achieve the same physiological and psychological effects. The second is physical dependence. Addiction implies an unwillingness to give up the drug. In this sense, virtually all psychoactive drugs are addictive. The depressants, however, produce physical as well as psychological dependence. After a period of regular use, stopping the drug precipitates a whole complex of "withdrawal" symptoms. These are always unpleasant and sometimes fatal.

Drug tolerance

Physical dependence on drugs

Volatile hydrocarbons

A number of volatile hydrocarbons are such effective depressants of the central nervous system that they can be used as anesthetics. They would have none but a medical interest if it were not for the fact that several of them (e.g. benzene, toluene) are too dangerous to be used in medicine but make excellent industrial solvents. They find widespread use as components of cleaning fluid, glues, and other materials. In a number

of countries, "sniffing" glue has become popular among very young people. It produces an effect like alcohol intoxication, and sometimes more. A number of cases have been reported where inhaling volatile hydrocarbons has suddenly caused death. Laboratory studies indicate that the cause may be a blockage in the transmission of the electrical impulse that triggers contraction of the ventricles (see Section 13.18).

The Hallucinogens

In moderate doses the hallucinogens have a powerful distorting effect on the subject's visual and auditory perceptions. There is also a marked enhancement of emotional responses. With higher doses, true hallucinations may occur—i.e. the subject "sees" and "hears" things that are not there at all. All the hallucinogens—mescaline, psilocybin, lysergic acid diethylamide (LSD), and dimethoxymethylamphetamine—share a striking similarity of chemical structure (Fig. 27.20). While their precise mode of action within the brain is still in question, it is certainly significant that their structure closely resembles that of serotonin, a natural component and presumed transmitter substance in parts of the brain. Dimethoxymethylamphetamine (STP) is also of interest in that it shares (as does mescaline) the structure and the properties of *both* the hallucinogens, e.g. LSD, *and* the amphetamines (Fig. 27.20).

Molecular mimicry

Marihuana

The place that marihuana should occupy in our long list of psychoactive drugs is uncertain. Until the isolation of its most active component, tetrahydrocannabinol (THC), little research had been carried out on the effects of marihuana. THC concentrates in certain areas of the brain where it has some of the milder effects of depressants like alcohol, and, in high doses, some of the perception-distorting effects of the hallucinogens. Unlike the other depressants, tolerance and physical addiction to THC do not occur. In fact, the drug is excreted so slowly from the body that, with repeated use, a given response is achieved with a lower dose.

As yet we have acquired only a tiny glimpse of the workings of the brain and the mechanisms by which psychoactive drugs exert their effects. As desirable as more such knowledge would be, we should not wait for it before searching for ways to reduce the enormous human waste that abuse of psychoactive drugs entails.

27.16 SUMMARY

In these chapters we have distinguished between chemical coordination and nervous coordination of body functions. Now we see that all coordination is really chemical. The only thing which differs is the means used to distribute the chemical. Hormones are transported throughout the body by the bloodstream. Transmitter substances are deposited in specific, localized spots by the action of the end plates of motor neurons.

Nerves accomplish the rapid, localized delivery of chemical regulators

By its very organization, the latter system permits more rapid, more specific, more variable body activity than the former. There is still another

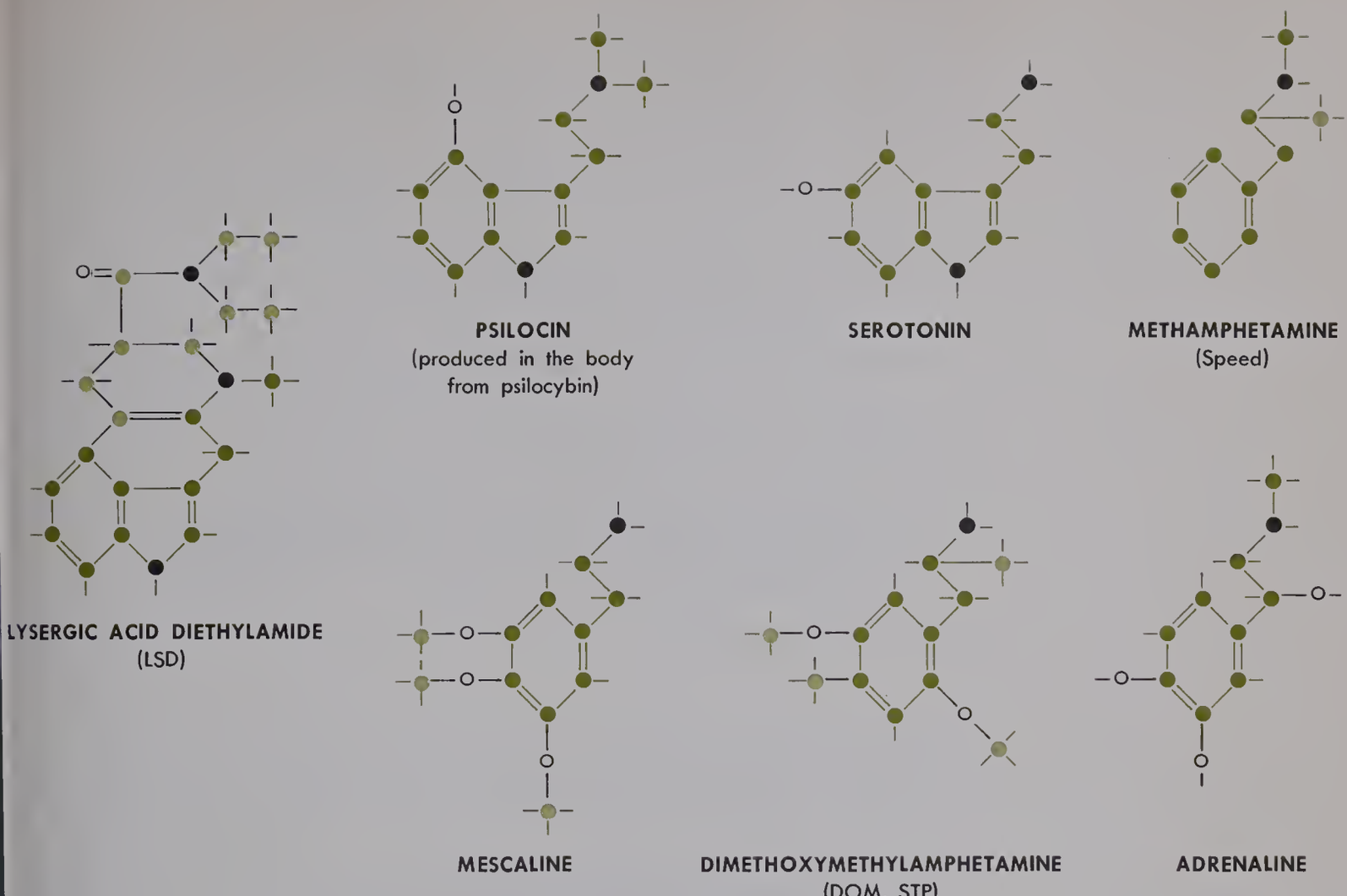


Fig. 27.20 Molecular mimicry may account for the effects of certain psychoactive drugs. The hallucinogens are similar in structure to serotonin, which is probably a transmitter substance in parts of the brain. The similarity of the amphetamines to adrenaline undoubtedly accounts for their ability to duplicate the effects of sympathetic nervous system activity. (Carbon atoms are shown in color, nitrogen atoms in black, and hydrogen atoms as short dashes.)

advantage to it. Localized release of transmitter substances by motor neurons permits the build-up of a higher concentration of the substance than could safely be tolerated by the body as a whole. It has recently been shown that the concentration of noradrenaline necessary to stimulate cells in the fatty tissue of laboratory mice and rats would be very harmful if allowed to circulate freely in the bloodstream. Its localized production by sympathetic neurons leading to the fatty tissue avoids this danger.

The body cannot respond to changes in its environment without the third essential feature of nervous coordination: the effectors. These are the structures that take action. Their morphology and physiology is the topic of the next chapter.

EXERCISES AND PROBLEMS

1. Meningitis is an inflammatory disease of what body structures?
2. What parts of the nervous system participate in the maintenance of balance and coordinated body movements?
3. Trace the route taken by nerve impulses arising when a withdrawal reflex is initiated.
4. The drug mecholyl stimulates parasympathetic nervous activity. What is its effect on (a) the pupil, (b) the salivary glands?
5. The drug atropine inhibits parasympathetic activity. Why is it used by ophthalmologists who wish to examine the interior of a patient's eye?
6. Damage in the left hemisphere of the brain interferes with the proper functioning of which side of the body?
7. Distinguish between cranial nerves and spinal nerves.
8. Describe the changes that occur in the body in times of emergency. Tell how each change helps prepare the body to cope with the emergency.
9. Distinguish between a nerve and a neuron.
10. What are ganglia?

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28 NERVOUS COORDINATION: THE EFFECTORS

Cytoplasmic Streaming

Cilia and Flagella

The Human Skeleton

The Structure of Skeletal Muscle

The Physiology of the Muscle Fiber

The Physiology of the Entire Muscle

The Chemistry of Muscular Contraction

Heat Production

Cardiac Muscle

Smooth Muscle

Electric Organs

Chromatophores

Luminescent Organs

When we say that all living organisms respond to stimuli, we mean that they do things when the external or internal environment changes in a significant way. The cells, tissues, or organs with which animals carry out actions are called effectors. The most important of the effectors are those which secrete substances and those which bring about motion.

Secretory effectors are glands. As we have noted previously, exocrine glands release the secreted material to the *external* environment through a duct. Endocrine glands release their secretions, hormones, into the *internal* environment. Glandular effectors are directly under either nervous control or chemical control or both.

28.1 CYTOPLASMIC STREAMING

Motion is a fundamental property of life. Plants, though they display no locomotion, do move in response to stimuli. These movements may be the result of unequal growth or sudden changes in turgor (Section 24.1). Even the contents of cells, especially plant cells, may be in motion. This phenomenon is known as cytoplasmic streaming. In plant cells, it usually occurs in a circular motion around the inner margin of the cell wall. Its function is probably the rapid distribution of materials throughout the cytoplasm. While the exact mechanism responsible for this motion is unknown, many microfilaments (Section 5.15) are found in streaming cytoplasm.

Dependence on microfilaments

In some cases, movement of cytoplasm within the cell may bring about locomotion of the cell. Both the amoeba and a white blood cell move by the streaming of their cytoplasm into **pseudopodia.** Energy in the form of ATP is needed, and proteins which contract in the presence of ATP have been isolated from the amoeba. However, the exact mechanism by which the energy is converted into motion is not yet known. (Several theories are examined in references No. 1 and No. 3 cited at the end of the chapter.) It seems to depend on changes in the properties of the cytoplasm. The cytoplasm in the interior of the amoeba, the "endoplasm," is watery. This endoplasm flows into the growing pseudopod (Fig. 28.1). As it does so, it is converted into a clear, semisolid "ectoplasm." At the rear of the cell, the opposite change takes place and thus the balance of endoplasm and ectoplasm is maintained. Probably it is the contraction of cytoplasmic proteins that brings the endoplasm forward into the pseudopodium, but just where the force is exerted remains to be established.

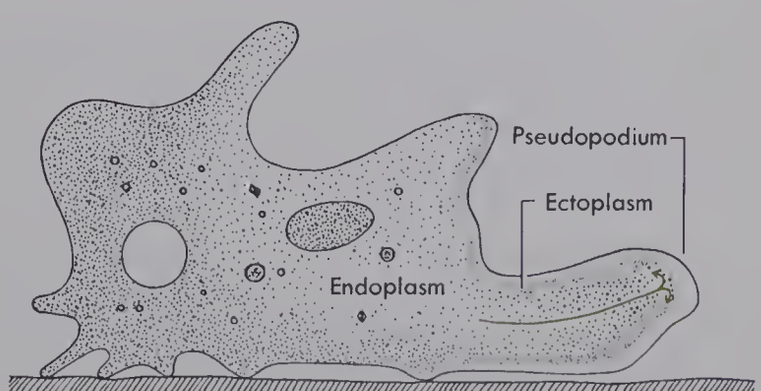


Fig. 28.1 Amoeboid movement. The clear layer at the tip of the pseudopodium is water that is freed when the endoplasm is converted into ectoplasm.

28.2 CILIA AND FLAGELLA

A number of protozoans and tiny aquatic animals move by means of many fine, hairlike cilia or one or more longer, whiplike flagella. Although cilia and flagella seem rather distinct in form, studies with the electron microscope reveal that they have the same basic structure and function in about the same way. Highly magnified cross sections of both cilia and flagella show that they are made up of a cylindrical array of long protein fibrils.

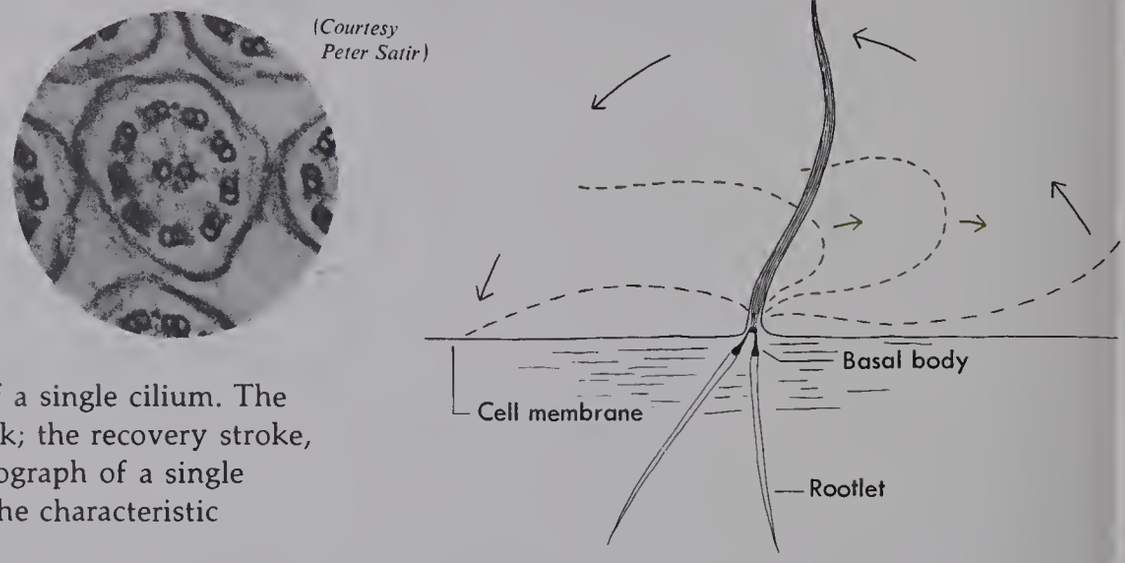


Fig. 28.2 At right, drawing of a single cilium. The power stroke is shown in black; the recovery stroke, in color. Above, electron micrograph of a single cilium in cross section. Note the characteristic pattern of fibrils.

The "9 + 2" arrangement of microtubules

Nine of these are evenly spaced around the periphery of the cylinder. Each appears to be a microtubule (See Section 5.16) with an accessory tubule attached to it. This gives them a "figure 8" appearance when viewed in cross section (Fig. 28.2). Running up through the center of the bundle are two fibrils, which are circular in cross section and identical to microtubules. The entire assembly of fibrils is sheathed in a membrane which is simply an extension of the cell membrane.

Basal bodies

Each cilium (and flagellum) is attached to a basal body embedded in the cytoplasm. Basal bodies are identical to centrioles (see Section 5.17) and are, in fact, produced by them. For example, one of the centrioles in developing sperm cells becomes a basal body and produces a flagellum after it has completed its role in the distribution of chromosomes during cell division (see Section 21.1).

No one yet knows just how cilia and flagella work. One theory that has been proposed is that an impulse starts in the basal body and passes up the central fibrils. This causes the outer fibrils on one side to contract rapidly, producing a "power stroke." Then a second impulse passes directly, but slowly, up the outer fibrils on the opposite side. This causes them to contract slowly, thus bringing the cilium back in a "recovery stroke" (Fig. 28.2). The motion of flagella is usually more complex, involving rippling and lashing movements. ATP is the source of energy for the motion of both cilia and flagella.

The sperm cells of all animals and even of many plants (mosses, for example) move by means of flagella. Ferns produce ciliated sperm cells. Ciliated epithelial cells are found in almost all animals. These cells are not free to move, but the beating of their cilia moves the ECF past them.

In man, cilia are present in the epithelial cells lining his air passages. A flagellum propels his sperm cells. Even movement by pseudopodia is found in his wandering white blood cells. However, the chief effectors in man for achieving motion are his muscles.

HUMAN MUSCLES AND HOW THEY WORK

Three kinds of muscle are found in vertebrates

Three distinct kinds of muscles are found in the human. **Skeletal** muscle is attached to the skeleton and permits locomotion. **Cardiac** muscle makes up the heart, and its contraction forces blood through the circulatory system. **Smooth** muscle lines the walls of all our other hollow organs and its contraction regulates the size of these organs.

Voluntary, directed body movements, such as those that occur when someone throws a ball or runs a race, would be impossible without the structural and functional coordination of both the skeletal muscles and the skeleton to which they are attached.

28.3 THE HUMAN SKELETON

The human skeleton is made up of cartilage and bone (Section 5.23, 2). Two hundred and six bones are found in most humans. These range in size from the tiny ossicles of the middle ear to the thigh bone or femur (Fig. 28.3).

Joints and ligaments

The bones of the skeleton are attached to one another at **joints,** most of which are held together by **ligaments,** a type of binding connective tissue. Motion of the skeleton is brought about by exertion of a muscular force across the joint. The motion of the lower arm illustrates this principle nicely. The upper-arm bone, the *humerus,* forms a "hinge" joint with one of the two lower-arm bones, the *ulna* (Fig. 28.4). The *biceps* muscle runs from the humerus to a second lower-arm bone, the *radius,* which is held parallel to the ulna. Thus, contraction of the muscle causes the arm joint to be bent. The biceps muscle is thus a *flexor.*

Antagonistic pairs of muscles

Because muscles can exert force only when contracting, not when relaxing, a second muscle, an *extensor,* is needed to straighten the joint. The *triceps* muscle, which extends from humerus to ulna, accomplishes this. Similar pairs of muscles, working antagonistically across joints, enable us to carry out almost all our skeletal movements.

The two ends of a skeletal muscle are not attached to the skeleton in the same way. Referring again to the triceps muscle, we see that the upper end, or **origin,** is attached directly to a large area of the humerus. The lower end, or **insertion,** tapers into a glistening white **tendon** which is attached

Tendons

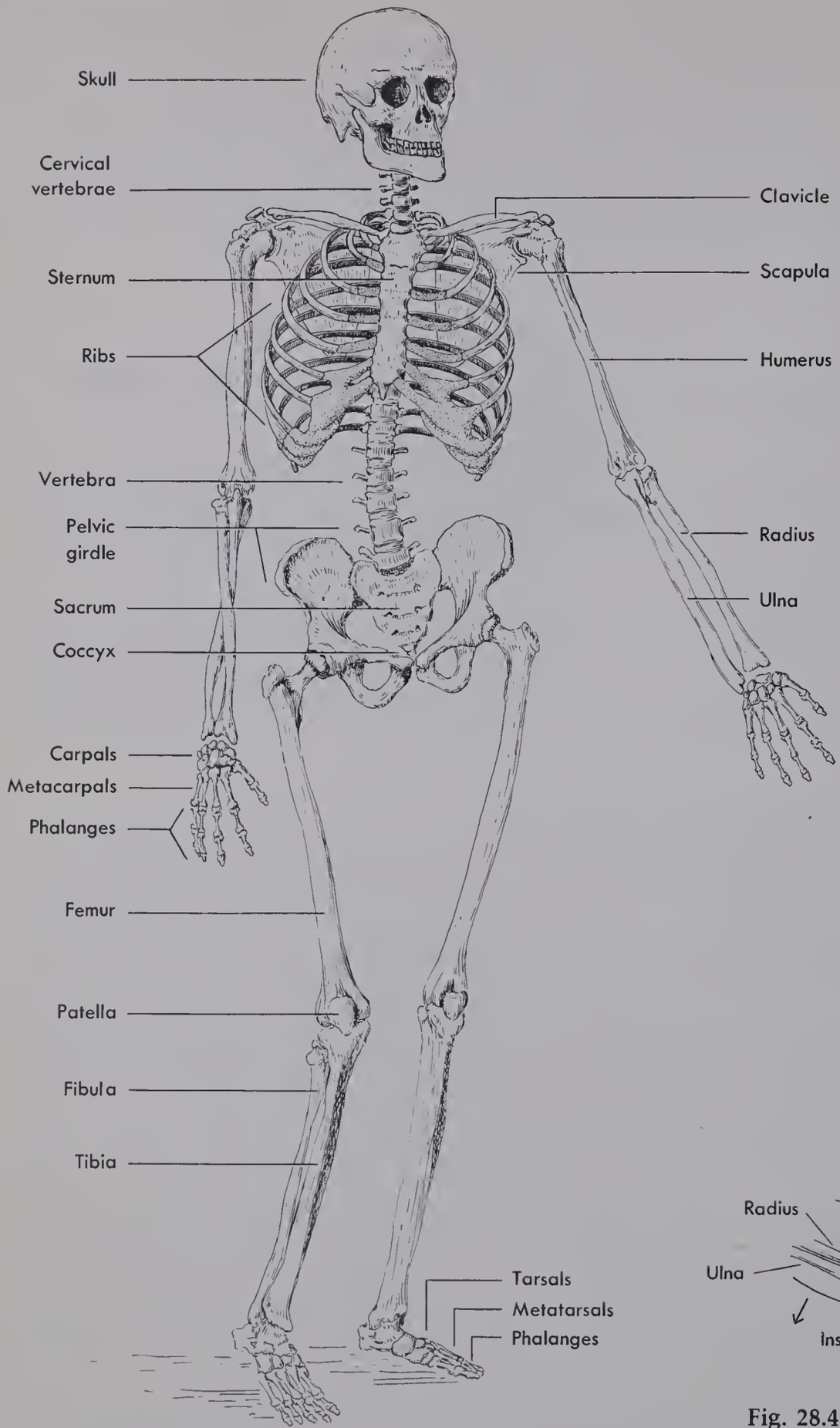


Fig. 28.3 The human skeleton.

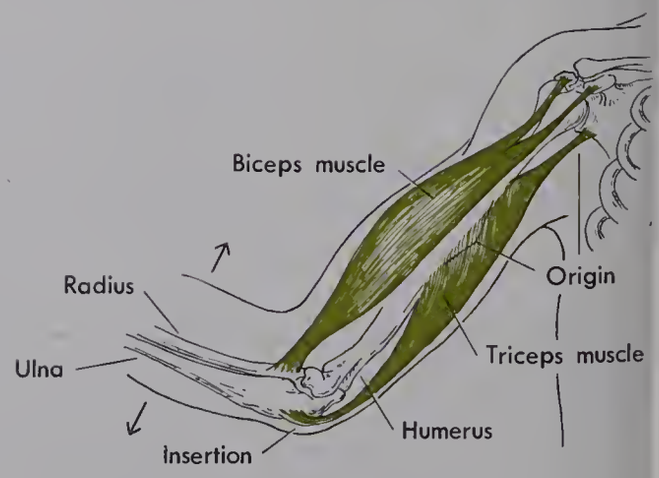


Fig. 28.4 Mechanism of movement of the human forearm. Antagonistic pairs of skeletal muscles accomplish movement at the various joints of the body.

to the ulna. During contraction, the origin remains stationary while the insertion does the moving. This pattern is found throughout the body.

Functions of the skeleton

Our skeleton has other functions besides motion. It provides a supporting framework for the soft organs of the body. For example, the bones of the pelvis support a large part of the weight of the viscera. The skeleton also provides protection against mechanical damage to delicate internal organs. Thus, the skull protects the brain, the spinal column of vertebrae protects the spinal cord, and the ribs and breastbone (sternum) protect the lungs and heart. Some of our bones (e.g. sternum, pelvis, ribs) possess a central, marrow-filled chamber in which red and white blood cells are manufactured.

28.4 THE STRUCTURE OF SKELETAL MUSCLE

A single skeletal muscle, such as the biceps muscle, consists of a thickened muscle *belly* attached at each end to bone. A cross section through the belly of the muscle reveals thousands of muscle **fibers** (Fig. 28.5). These are arranged in parallel bundles and may extend uninterrupted from origin to insertion. The fibers are bound together by connective tissue through which blood vessels and nerves also run. The number of fibers in a muscle is probably fixed. Increased strength and size of the muscle is produced by an increase in the thickness of the individual fibers and an increase in the amount of other tissue, such as blood vessels and connective tissue, in the muscle.

The muscle fiber

Seen from the side, the muscle fibers show a pattern of cross-banding or striations. This banded appearance gives rise to another common name for skeletal muscle: striated muscle.

Although they may be many inches long, single muscle fibers range from only about 10 μm to 100 μm in diameter. A magnified cross section of a single muscle fiber reveals that it is packed with **myofibrils** in much the same way the muscle belly was packed with muscle fibers. The myofibrils are all stacked lengthwise and seem to run the entire length of the fiber. The myofibrils are embedded in a liquid cytoplasm which also contains many nuclei, mitochondria, and an extensive endoplasmic reticulum. The presence of many nuclei is a consequence of the fact that during embryonic development each muscle fiber is formed by the fusion of a number of individual cells. Strictly speaking, then, the muscle fiber is not really a cell but a syncytium (see glossary). For this reason, its parts are often given special names (e.g. sarcolemma for cell membrane, sarcoplasmic reticulum for endoplasmic reticulum), although this tends to obscure the essential similarity in structure and function of these structures and those that are found in "true" cells.

Myofibrils

The nuclei and mitochondria are located just beneath the cell membrane (Fig. 28.5). The endoplasmic reticulum extends between the myofibrils.

The pattern of striations in a muscle fiber arises from the striations present in the individual myofibrils. Normally, these are all in register with

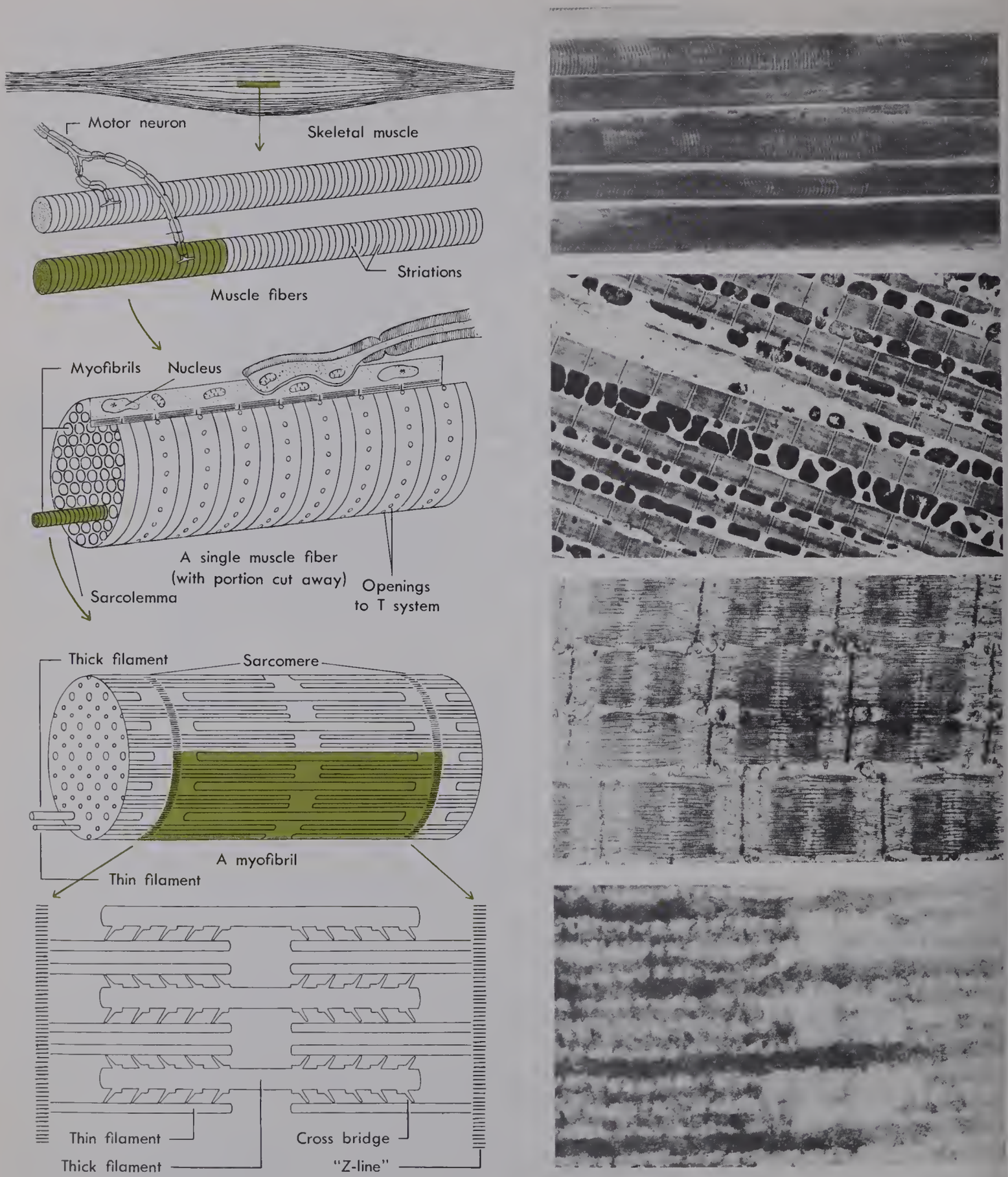


Fig. 28.5 Organization of skeletal muscle at various levels of magnification. The pictures on the opposite page are cross sections corresponding roughly to the drawings on the left. The pictures above are corresponding longitudinal views. (All electron micrographs courtesy of Dr. H. E. Huxley.)

one another, although in specimens prepared for electron microscopy, the parallel registration is usually disrupted.

With the electron microscope, it is now possible to examine the structure of an individual myofibril. In cross section, these are seen to be made up of a neat geometrical array of **filaments.** These filaments are of two sizes, thick and thin, the former about 15 nm and the latter about 5 nm in diameter. Chemical analysis of the myofibrils indicates that the thick filaments are made up of a protein, **myosin,** while the thin filaments are made up of a second protein, **actin.**

Longitudinal examination of myofibrils shows that the filaments do not extend the entire length of the myofibril but are stacked in repeating arrays, called **sarcomeres,** that are arranged end-to-end throughout the length of the myofibril. The boundaries of the sarcomeres (called the "Z-lines") and the overlapping of the thick and thin filaments produce the pattern of striations characteristic of the myofibrils and, for that matter, of the whole fiber. It is easy to see that less light will pass through the portions where the thick and thin filaments overlap than where they do not.

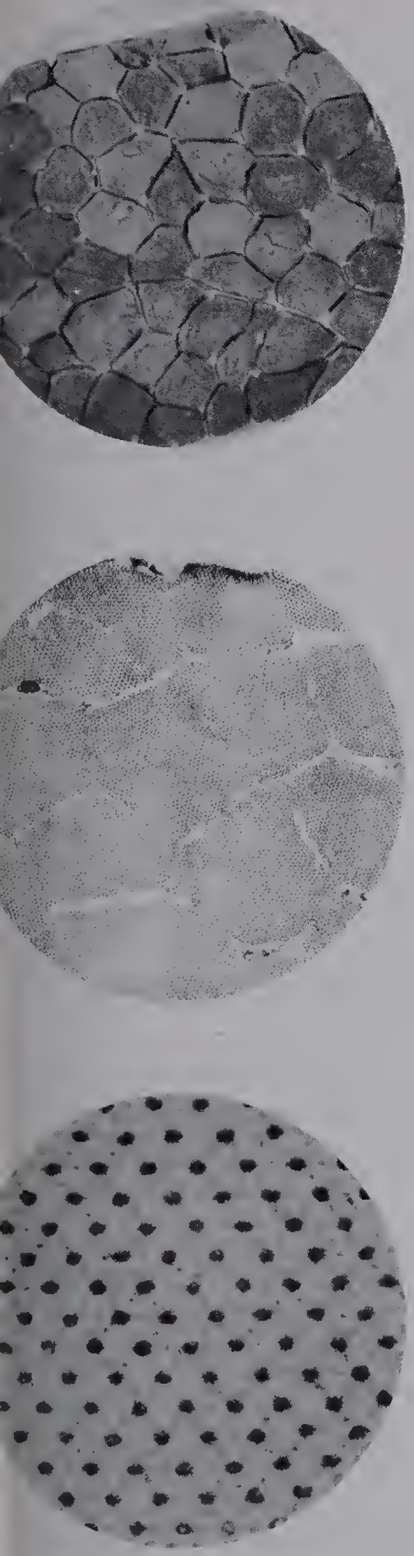


Fig. 28.5 (cont.)

28.5 THE PHYSIOLOGY OF THE MUSCLE FIBER

The exterior of the skeletal muscle fiber, like that of the neuron, is positively charged (about 0.1 volt) with respect to the interior. This polarization of the membrane is maintained by the active transport of Na^+ and K^+ ions just as in the neuron. Electric shocks and certain chemical substances are capable of reducing this polarity which, again like the neuron, increases the permeability of the membrane to Na^+ ions. If the stimulus has sufficient strength, the threshold of the fiber is reached and a wave of depolarization, the **action current,** sweeps along the length of the fiber. The response is "all-or-none" in that the fiber either fires or does not. A stimulus greater than threshold produces no greater response than a threshold stimulus.

No visible change takes place in the muscle fiber during the period in which the action current is sweeping along it. Hence, this period which may be from 3 to 10 msec long, depending on the particular fiber studied, is called the **latent period.** During this time, the action current also penetrates the interior of the fiber. This is important because the efficient contraction of the muscle fiber requires that all its myofibrils receive the signal to contract at approximately the same time. Spaced evenly along and around each fiber are inpocketings of its membrane which carry the action current into the interior. This is known as the T system (Fig. 28.5). The tubes of the T system terminate at or near the "Z-line" of the myofibrils, that is, at the boundaries of each sarcomere. The endoplasmic reticulum is particularly well developed in this region, and it is thought that the arrival of the action current triggers the release of calcium (Ca^{++}) ions by the endoplasmic reticulum. The Ca^{++} ions, in some way, cause contraction of the sarcomeres. Thus the entire fiber contracts. Over a period of about

50 msec, the fiber shortens, exerting a force if there is any resistance offered to its shortening. While the fiber is contracting, the pattern of light and dark striations is altered, indicating that the thick and thin filaments in the myofibrils are sliding over one another. Chemical studies confirm that actin and myosin link together during contraction of the fiber, and the electron microscope shows cross bridges between the two kinds of filaments. The protein molecules of these cross bridges may actually contract, pulling the actin and myosin filaments past each other in a ratchetlike action (Fig. 28.5). In any case, we know that the individual *filaments* do not shorten, thicken, or fold during the process.

Sliding filaments

Contraction of the muscle fiber (which is also all-or-none) is automatically followed by relaxation if the fiber is not stimulated again. The fiber takes about 50 msec to resume its original length. However, the fiber will not relax if it is stimulated again before the relaxation period begins. There is only a 7-msec **refractory period** after the action current sweeps down the muscle fiber. Then the polarity of the membrane is restored by the passage of K^+ ions out of the cell, and the fiber is ready to be stimulated again. Note that the refractory period is much shorter than the time it actually takes the fiber to contract and relax. This means that the fiber can be maintained in the contracted state so long as it is stimulated frequently enough (50 stimuli/sec does very nicely). Such sustained maximal contraction is called **tetanus.** As we normally use our muscles, the individual fibers usually go into tetanus for brief periods rather than simply undergo single twitches.

Tetanus

The neuromuscular junction

The stimulus that depolarizes the skeletal muscle fiber is ACh released at the neuromuscular junction (see Section 27.11). This acts upon the muscle fiber membrane, causing its depolarization. If sufficient ACh is released, the depolarization exceeds the threshold value and an action current is generated.

Once the ACh has done its job, it must be removed in order to prevent permanent tetanus of the muscle fiber. This is accomplished by the enzyme acetylcholinesterase, which quickly breaks the ACh down into inactive products and clears the field of action.

Anything which interferes with the action of acetylcholinesterase causes a serious disturbance of muscular activity. A great many substances have been discovered which do just this and, in every case, they are quite poisonous. Organophosphate insecticides, such as parathion and the closely related "nerve gases," function in this way. Exposure to toxic amounts of these substances leads to convulsions, paralysis, and often death.

In man, motor neurons have only a stimulating effect on muscular contraction. Inhibition of muscles occurs as a result of preventing nerve impulses in the central nervous system from reaching specific motor neurons. In the crayfish, however, motor neurons have been found which release a hyperpolarizing substance at the neuromuscular junction, thus inhibiting contraction of the muscle fiber. It should be clear that the prop-

erties of the neuromuscular junction and the synapse are very much alike. Just as the synapse can become fatigued through repeated use, so can the neuromuscular junction, but fatigue at this site does not appear as quickly as it does at the synapse.

28.6 THE PHYSIOLOGY OF THE ENTIRE MUSCLE

Although the individual muscle fiber is the *structural* unit of skeletal muscle, it is not the *functional unit.* All motor neurons leading to skeletal muscles have branching axons, each of which terminates in a neuromuscular junction with a single muscle fiber. Nerve inpulses passing down a single motor neuron will thus trigger contraction in all the muscle fibers at which the branches of that neuron terminate. This minimum unit of contraction is called the **motor unit.** For some muscles over which we have very precise control (for example, the muscles controlling eye movements) a single motor neuron may trigger only two to six muscle fibers. For other muscles (e.g. the biceps) over which our control is less precise, the motor units may include several hundred muscle fibers (scattered fairly uniformly through the muscle belly).

The motor unit

We have seen that the response of a single muscle fiber is all-or-none. Nevertheless, we know that an entire muscle does not behave in this fashion. It is possible to contract a muscle any desired degree from practically relaxed to maximally contracted. This can be demonstrated in the laboratory by stimulating the calf muscle (gastrocnemius) of a frog with an electrical stimulator and measuring, by means of a writing lever (Fig. 28.6), the amount of contraction of the entire muscle. Too weak a shock will have no effect at all. When threshold is reached, the muscle twitches slightly. Then as the strength of the stimulus is increased, the amount of contraction increases up to a maximum. Still greater stimuli are no more effective (Fig. 28.6). How can we reconcile this graded response of the entire muscle with the all-or-none properties of the individual fibers which make it up? The answer is that the strength of contraction of an entire muscle increases as the number of individual contracting fibers increases.

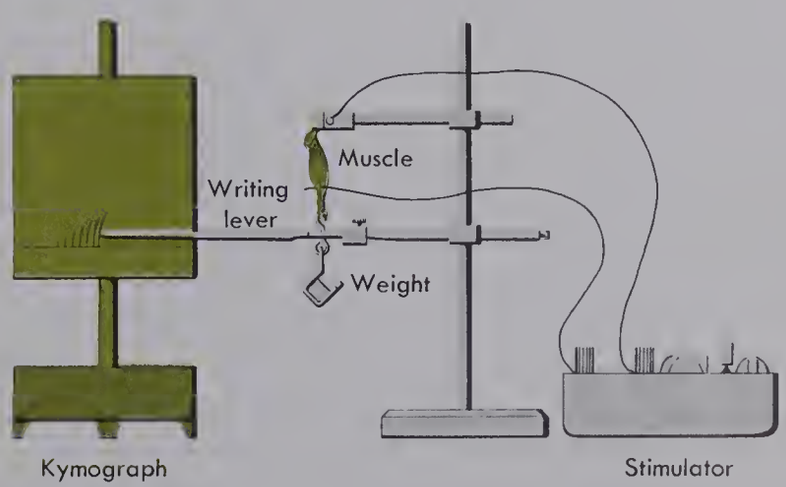


Fig. 28.6 Measuring the response of the calf muscle (gastrocnemius) of a frog to single shocks of increasing voltage. Contraction of the muscle lifts the writing lever, which makes a mark on the paper wrapped around the kymograph drum.

Thus, in the intact animal, the strength of the muscular response is controlled by the *number of motor units* activated by the central nervous system.

Tonus

Even at rest, most of our skeletal muscles are in a state of partial contraction called **tonus.** If this were not so, we would have a very difficult time maintaining good posture. The action of the motor units provides us with a physical basis for tonus. A few motor units are activated at all times even in the resting muscle. As one set of motor units relaxes, another set takes over. This synchronous activation and deactivation of a small percentage of the total number of motor units maintains the tonus of our skeletal muscles.

28.7 THE CHEMISTRY OF MUSCULAR CONTRACTION

Muscular contraction produces heat and may perform work. It therefore cannot occur without a source of energy. The energy comes from the same source as the energy for all other types of cellular work: the high energy phosphate bonds of ATP.

ATP and actomyosin

Contraction of muscle fibers requires actin, myosin, ATP, Ca^{++}, and Mg^{++} ions. Even in the test tube, actin and myosin strands can be made to contract when supplied with ATP and traces of the necessary ions. The exact chemical mechanism is not fully understood, but the passage of the action current through the T system leads to the release of Ca^{++} ions by the endoplasmic reticulum and the union of actin and myosin to form actomyosin. Actomyosin is a powerful ATPase, that is, it is an enzyme that splits the third phosphate group from the ATP molecule. This leaves actomyosin and ADP and a phosphate group. In some way, the energy initially stored in the high-energy phosphate bond is used to provide the energy for contraction. Then the Ca^{++} ions are taken back into the endoplasmic reticulum, the actin and myosin become separated, and the ADP is resynthesized into ATP.

Very little ATP is stored in the muscle fiber. A burst of muscular activity quickly (after a few twitches) exhausts the supply. Additional ATP can be supplied by cellular respiration, but if violent, continued muscular activity is called for, the output from this source will be temporarily unable to meet the demand. This is because there is a delay in the increase of the rate and strength of heartbeat, the rate and depth of breathing, and hence in the transport of additional supplies of oxygen to the muscle. Fortunately, our muscles are equipped to carry on successfully during this lag period. Each muscle fiber contains a supply of **creatine-phosphate.** This substance contains a phosphate group attached by a high-energy bond and thus can serve as a reservoir of $\sim$ Ⓟ (Fig. 28.7). The creatine-phosphate serves to resynthesize ATP by giving up its high-energy phosphate to ADP. This mechanism enables muscles to work at the maximum rate for about 15 sec. During violent effort, however, even 15 sec is not enough time for the heart and lungs to adapt to the oxygen requirements of the muscles. To bridge the gap, the muscle fibers manufacture ATP by fermentation. Glycogen stored in the muscle is broken down

The role of creatine phosphate

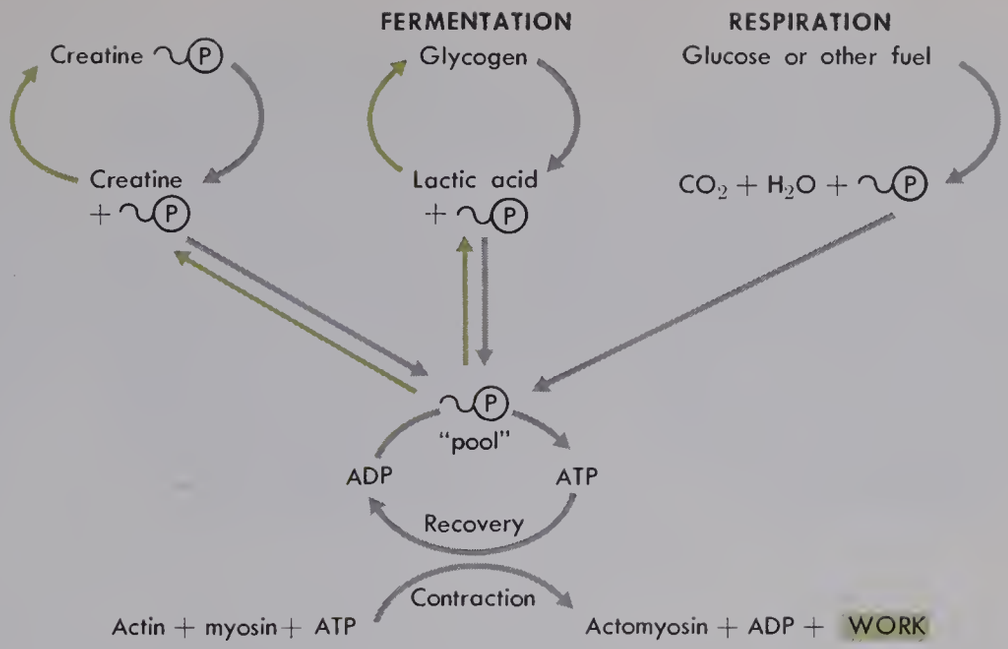


Fig. 28.7 The chemistry of muscular contraction. (Pathways in color function during payment of oxygen debt.)

Lactic acid is produced by fermentation

into lactic acid by the mechanism we discussed in Chapter 8. Although this process (which takes place in the endoplasmic reticulum) produces ATP and thus enables the muscle to keep functioning, it is only a temporary expedient. As we have noted, fermentation is an extremely inefficient method of energy production. Most of the potential energy of the glycogen remains trapped in the lactic acid. Furthermore, the accumulation of lactic acid lowers the pH of the muscle fluids. If the pH shift is too great, the muscle fiber loses its ability to contract, thus becoming *fatigued.* A general lowering of the pH of the ECF may produce serious consequences elsewhere in the body, too.

Therefore, protracted muscular exertion, such as that which occurs in running or swimming a distance race, requires that the heart and lungs quickly supply sufficient oxygen to the muscles to enable ATP to be produced by cellular respiration. When the race is finally over, we all know that the rates of breathing and heartbeat do not drop back to normal levels immediately. First, the **oxygen debt** must be paid. This is the quantity of oxygen which was needed by the muscles for respiration during the early part of the race but was not supplied. In chemical terms, it is measured by the accumulation of creatine and lactic acid during that period. Lactic acid leaves the muscle cells (by way of the intracellular channels provided by the endoplasmic reticulum) and is carried in the blood to the liver. Here it is resynthesized into glycogen using energy from cellular respiration. Continued high levels of cellular respiration in the muscles themselves supply the ATP needed to resynthesize creatine phosphate. Later, muscle glycogen is restored by transport of glucose from the liver to the muscles.

Repayment of the oxygen debt

28.8 HEAT PRODUCTION

The conversion of the chemical energy of ATP into the mechanical energy of muscle contraction is, at best, no more than 40% efficient. The remain-

ing energy is liberated as heat, which is, however, a valuable by-product. Birds and mammals are homeothermic; that is, they are able to maintain the temperature of their ECF practically constant despite wide fluctuations in the temperature of the external environment. Even with the insulation provided by feathers and fur, these creatures lose heat to the environment whenever its temperature falls below that of their body. The process can be and is slowed by shutting down the capillary beds of the skin, thus cutting down the radiation of heat from the blood. To replace lost heat, however, the homeothermic organism depends primarily on the heat produced during muscular contraction. In fact, shivering is an involuntary muscular response during which virtually all of the energy of ATP is being converted into heat. The goose flesh that may appear on our skin at the same time is simply a now-useless response which, in our hairy ancestors, served to thicken the layer of fur and thus improve its insulating qualities.

Maintaining homeothermy

Too great a production of heat in warm surroundings brings about reactions that aid in cooling the body. The capillary beds of the skin become filled with blood which is thus able to radiate the heat transported from the internal organs. The sweat glands secrete sweat which, as it evaporates, absorbs heat from the body, thus cooling it. Man is unusual among the mammals in possessing so many sweat glands scattered over the surface of his body. These probably compensate for his lack of body hair which, after all, is just as effective at keeping heat out as keeping it in.

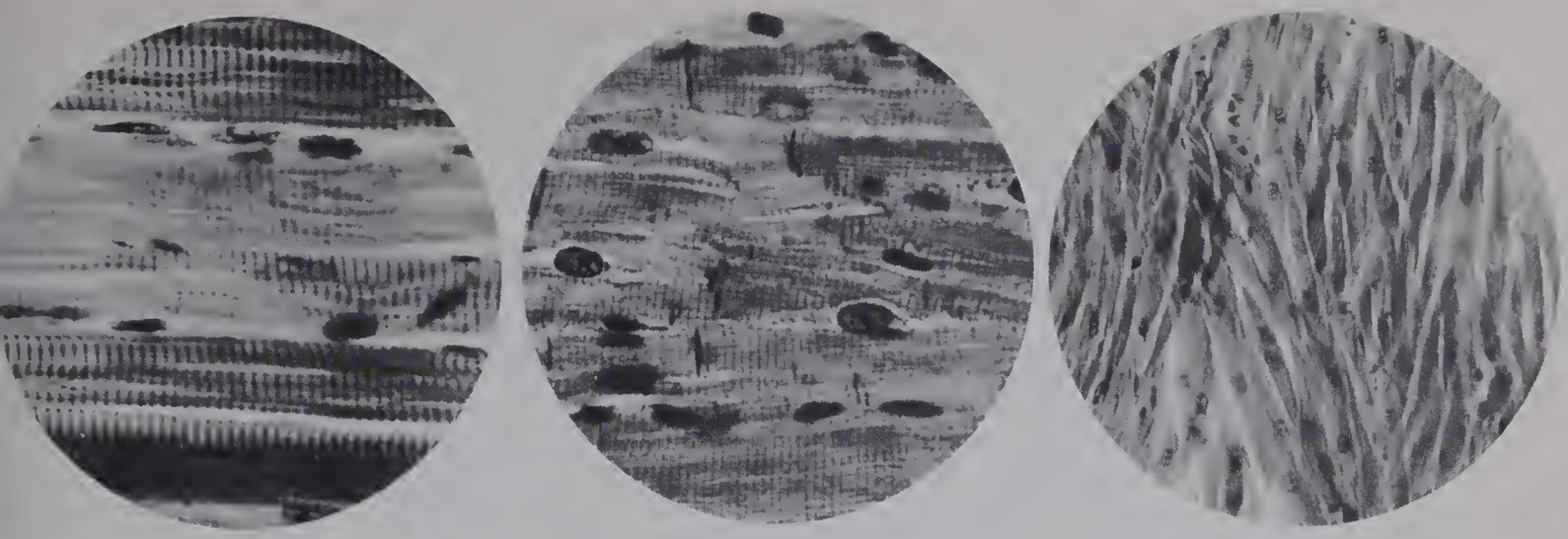
The various responses of the body to heat and cold are regulated by the autonomic nervous system. The controlling impulses originate in the hypothalamus where the temperature of the blood is monitored constantly. The heat and cold receptors of the skin may also play a minor role in initiating these responses.

28.9 CARDIAC MUSCLE

Cardiac muscle is made up of interlocking, striated fibers (Fig. 28.8). If you look back at Fig. 13.12, you will see that the myofibrils run roughly parallel to one another and that the organization of the sarcomeres is like that found in skeletal muscle. However, the myofibrils in cardiac muscle are often branched and mitochondria are more abundant than in our skeletal muscle fibers.

Contraction of cardiac muscle does not depend on stimulation by nerves

Cardiac muscle differs from skeletal muscle in its physiology as well. The impulse which causes cardiac muscle to contract is self-generated. It is true that sympathetic and parasympathetic nerves run to the heart, but their control is purely accessory. Even when they are destroyed, the heart continues to beat as long as glucose and oxygen are available. Any deprivation of oxygen, such as that which occurs in a coronary occlusion (see Section 13.8) quickly results in death of the portion of heart muscle so deprived. Cardiac muscle, unlike skeletal muscle, has a longer refractory period than relaxation period. Hence, no tetanus is possible.



SKELETAL CARDIAC SMOOTH

Fig. 28.8 The three kinds of muscle as they appear under the light microscope. Skeletal and cardiac muscle are made up of long, multinucleate fibers. Smooth muscle is made up of single, spindle-shaped cells. (Skeletal muscle courtesy of Ward's Natural Science Establishment, Inc. Others, courtesy of the General Biological Supply House, Inc.)

28.10 SMOOTH MUSCLE

Each smooth muscle fiber is a single spindle-shaped cell with a single nucleus (Fig. 28.8). The cells are arranged in sheets. Smooth muscle is also nonstriated muscle because no pattern of cross striations can be seen under the light microscope. However, the electron microscope has revealed that the cell contains filaments of both actin and myosin. How they produce the force of contraction is not yet known.

Smooth muscle is controlled by the autonomic nervous system

Smooth muscle may contract spontaneously, but it is primarily controlled by the motor neurons of the sympathetic and parasympathetic nervous systems. As we have seen, parasympathetic postganglionic neurons release ACh at their end plate just as the motor neurons of the sensory-somatic system do. Sympathetic postganglionic neurons usually release noradrenaline. The effect of these substances on a given piece of smooth muscle varies with the particular muscle but is always antagonistic. If ACh stimulates the muscle, noradrenaline inhibits it and vice versa. For example, ACh stimulates contraction of the muscular wall of the intestine while noradrenaline inhibits it. On the other hand, ACh inhibits the contraction of the muscular walls of the arterioles supplying blood to the viscera while noradrenaline stimulates their contraction.

The action of smooth muscle is much slower than that of skeletal muscle. It may take anywhere from three seconds to three minutes for it to contract. Furthermore, smooth muscle differs from skeletal muscle in its ability to remain contracted at varying lengths. This state is also called

tonus, but unlike tonus in skeletal muscle, it does not seem to require continual stimulation and energy production.

Smooth muscle lines the walls of all our hollow organs and vessels. Its contraction regulates the size of these structures and thus controls the passage of materials through them. Constriction of arterioles, emptying of the bladder, peristalsis in the alimentary canal, even childbirth all depend upon the special contractile properties of smooth muscle.

Smooth muscle is found exclusively in many invertebrates. The earthworm, for example, accomplishes all its body movements, including locomotion, with it alone.

OTHER EFFECTORS

Other effectors besides glands and locomotor structures are found here and there in the animal kingdom.

28.11 ELECTRIC ORGANS

Certain fishes (for example, the South American electric eel) possess electric organs. These are masses of flattened cells, called electroplates, which are stacked in neat rows along the sides of the animal. The posterior surface of each electroplate is supplied with a motor neuron; the anterior surface is not. At rest, the interior of each electroplate, like a muscle or nerve cell, is negatively charged with respect to the two exterior surfaces. The potential is about 0.08 volt but, because the charges alternate (Fig. 28.9), no current flows. When a nerve impulse reaches the posterior surface, however, its polarization reverses. It is believed that the liberation of ACh by the neuron increases the permeability of the posterior membrane to the inflow of Na^+ ions so that a momentary reversal of charge occurs as in the action current in nerves and muscles. (In most fishes, electroplates are, in fact, modified muscle cells.) The anterior surfaces remain positively charged but the posterior surfaces become negatively charged. The charges now reinforce each other and a current flows just as it does through an electric battery with cells wired in "series." Voltages as high as 650 volts are produced by the several hundreds of thousands of electroplates in the South American electric eel. The flow (amperage) of the current is sufficient (¼–½ ampere) to stun a man if not kill him. The pulse of current can be repeated several hundred times each second.

Physiology of electric organs

Functions of electric organs

Electric organs are probably used both as offensive and defensive weapons. Prey can be stunned for easy consumption. There is evidence that some fishes with electric organs too weak to use as weapons use them instead to detect the presence of other objects, like predators or prey, in murky water. Such objects probably alter the conduction of electricity through the water, and this alteration is detected by special electric *recep-*

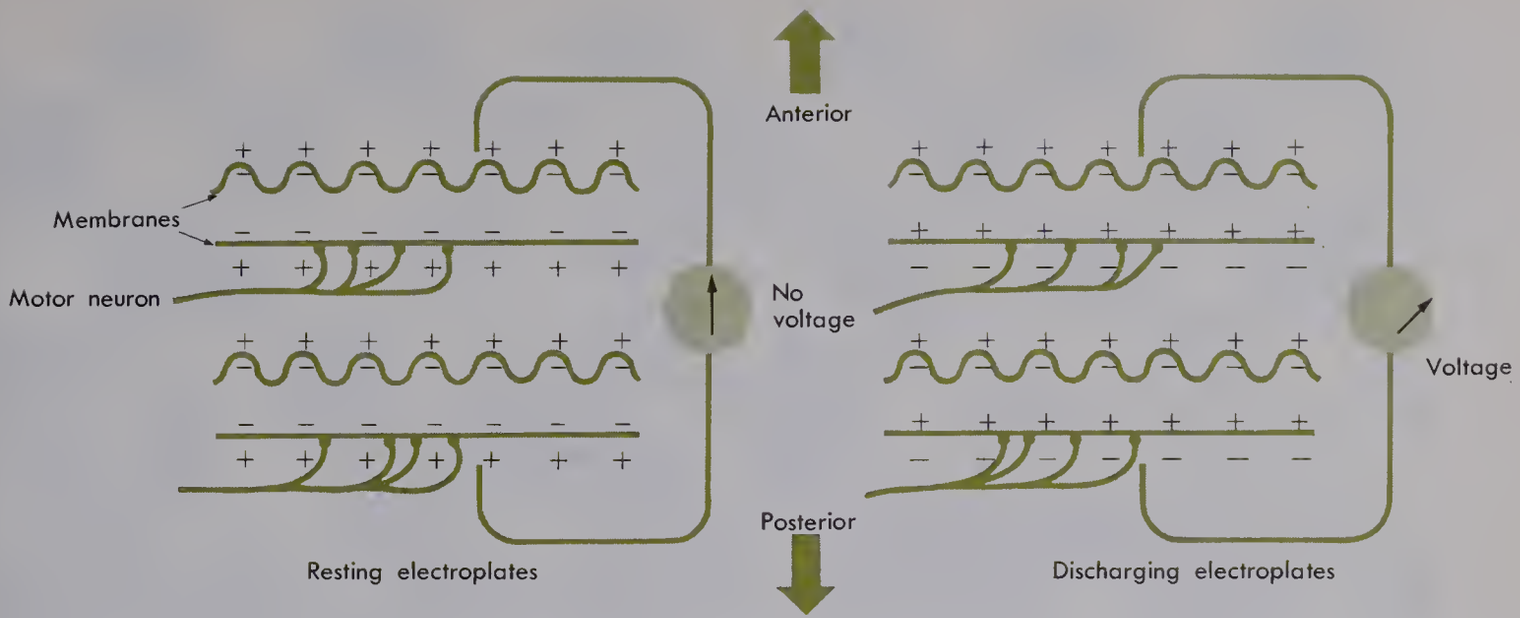


Fig. 28.9 Method of action of the electroplates in the South American electric eel. Nerve impulses reaching the posterior membranes reverse their potential and cause each electroplate to function like a cell in a battery. With several thousand of these cells arranged in "series," voltages as high as 650 volts are produced.

tors. Some electric fishes may also use their electric transmitters and receivers to locate mates. In one case, the discharge frequency of the females differs from that of the males and stimulates courtship behavior in the males. Not only do electric fishes have receptors for detecting electric currents in the water, but at least one species that has no electric *organs* does have electric receptors. Perhaps it uses these to detect the approach of electric fishes!

28.12 CHROMATOPHORES

Chromatophores are purely defensive effectors. They are irregularly shaped, pigment-containing cells such as the melanophores of the frog (Fig. 25.8). By changing the distribution of pigments in these cells, the animal is able to alter its body color and blend into its surroundings. Figure 28.10 shows how remarkably effective this mechanism can be. Many experiments have shown that these protective changes in body coloration require the presence of eyes and nervous system. However, motor neurons do not activate the chromatophores directly in amphibians and crustaceans. Instead, hormones transmit the stimulus from the central nervous system to the chromatophores.

28.13 LUMINESCENT ORGANS

Many marine animals give off visible light. This is particularly common in those species which live in the dark depths of the ocean. In some cases,

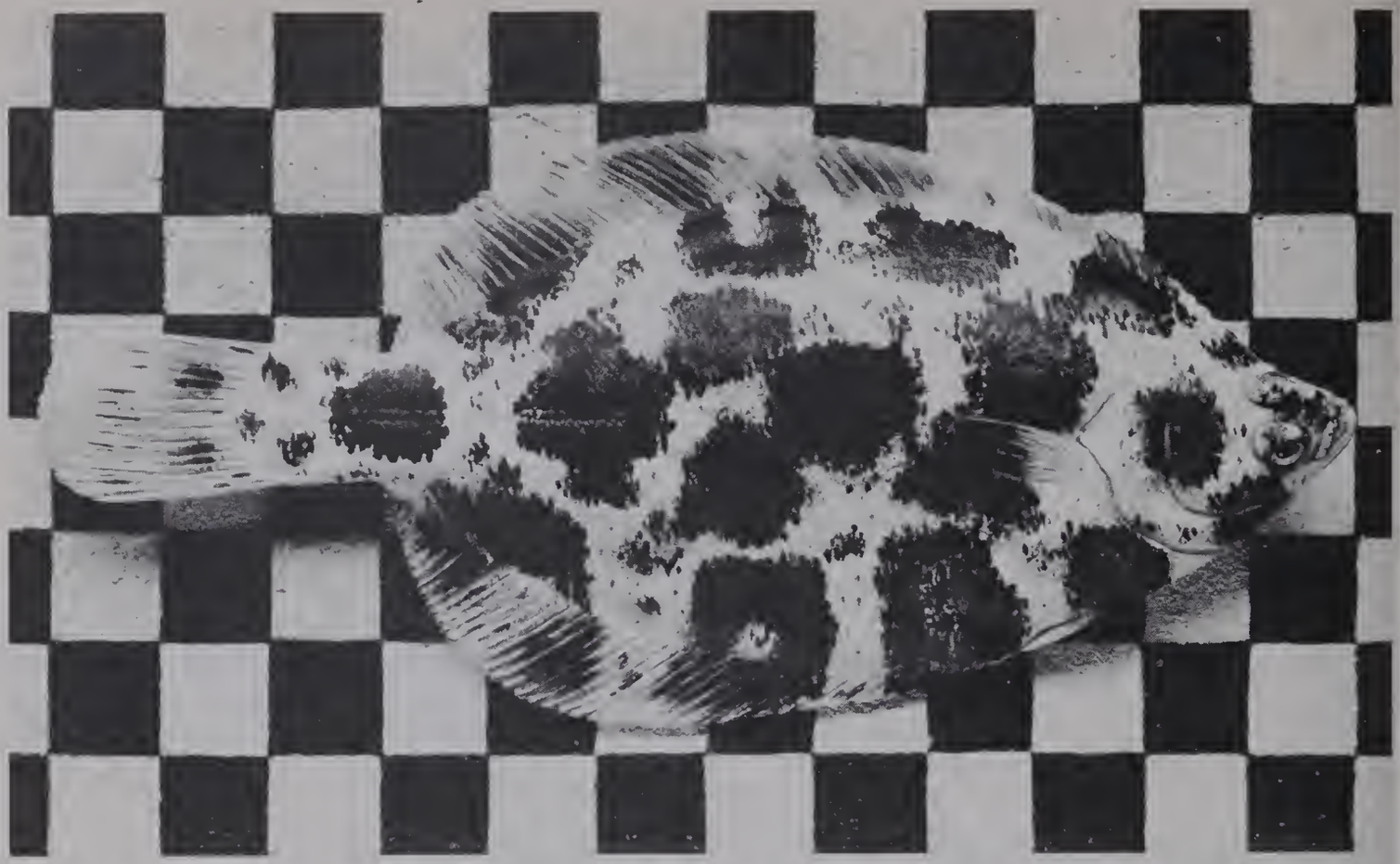


Fig. 28.10 This winter flounder resting on a checkerboard pattern shows how versatile is its use of chromatophores for camouflage. (Courtesy of the Field Museum of Natural History.)

special luminescent organs emit the light. In others, the light is produced by luminescent bacteria which adhere to the animal's body.

ATP is the energy source for bioluminescence

Most of us are more familiar with the luminescence of the firefly. The periodic flashes of light which we see so frequently on warm summer evenings are produced by special luminescent organs in the abdomen of the insect. The mechanism by which the light is produced is now fairly well understood. It involves a chemical **(luciferin),** an enzyme **(luciferase),** oxygen, and ATP (Fig. 28.11). The more ATP available, the brighter the light. In fact, at least one manufacturer of biochemicals sells ground firefly "tails" to be used for determining the concentration of ATP in different biological materials. The ATP-containing material is added to a carefully prepared extract of the firefly tails, and the amount of light given off is measured with a sensitive light meter. The company itself is kept supplied with frozen fireflies by youngsters in the southern part of the United States who earn extra money by trapping the insects and selling them.

Functions of luminescent organs

The widespread occurrence of luminescent organs among deep-sea animals reflects the perpetual darkness in which these creatures live. At least one fish has its luminescent organ located at the tip of a protruding stalk and uses it as bait to lure prey within reach of its jaws. When

disturbed, one species of squid emits from its mantle cavity a cloud of luminous water instead of the protective smokescreen of ink that its shallow-water relatives employ. Some marine animals living nearer the surface have their luminescent organs on their underside. These probably make it more difficult for predators lurking beneath to see them against the light background of the surface. Luminescent organs also serve to attract mates and thus assure that eggs and sperm will be released close to one another.

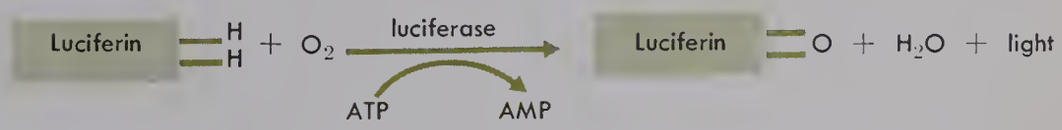


Fig. 28.11 Chemistry of luminescence in the firefly. (AMP = adenosine monophosphate. Subterminal ~ bond is used.)

It has been well demonstrated that male and female fireflies attract each other with their flashing lights. The pattern of flashing differs from species to species. In one species, the females sometimes mimic the pattern used by the males and females of another species. When the males of the second species respond, they are eaten!

Receptors, conductors, effectors: these are the structures which enable animals to detect changes in their environment, both external and internal, and to respond in appropriate, coordinated ways to these changes.

When these responses are directed externally, as in motion or locomotion, we say that the organism is displaying behavior. Some of the varied ways in which organisms behave will be considered in the next chapter.

EXERCISES AND PROBLEMS

1. Outline the chemical changes that occur in the leg muscles of a mile runner from the time the starter's gun is fired until the runner is completely recovered.
2. Why is the dangerous disease "lockjaw" known scientifically as *tetanus?*
3. What kind of muscle lines the walls of arterioles?
4. List the various functions carried out by the skeleton and give a specific example of bones that carry out each function.
5. What is the structural unit of the biceps muscle?
6. What is the functional unit of the biceps muscle?
7. How does fermentation in skeletal muscles differ from yeast fermentation?
8. In what ways does the physiology of a skeletal muscle fiber resemble that of a motor neuron? In what ways does it differ?
9. Although most birds are homeothermic, the body temperature of hummingbirds drops at night. Can you suggest an explanation for this?
10. Summarize the cell activities that are energized by ATP.

11. Distinguish between (a) skeletal muscle, (b) cardiac muscle, and (c) smooth muscle on the basis of both structure and function.
12. Distinguish between tetanus and tonus in skeletal muscles.
13. How can you reconcile the graded response of skeletal muscles with the all-or-none law?
14. In what ways does the physiology of the electroplates of electric organs resemble that of skeletal muscle?

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29 BEHAVIOR

What is Behavior?

Behavior in Plants

Taxes

The Simple Reflex

Instincts

Releasers of Instinctive Behavior

Rhythmic Behavior and Biological "Clocks"

The Life History of the Honeybee

The Work of the Hive

Receptors, Conductors, and Effectors in the Honeybee

Communication Among Honeybees

Habituation

Imprinting

The Conditioned Response

Instrumental Conditioning

Concepts/Language/Memory

29.1 WHAT IS BEHAVIOR?

Behavior is action which alters the relationship between the organism and its environment. It is externally directed activity and does not include the many internal changes which are constantly taking place in living things.

Behavior may occur as a result of an external stimulus. Receptors are necessary to detect the stimulus, conductors are needed to coordinate the response, and the effectors actually carry out the action. Behavior may also occur as a result of an internal stimulus. A hungry animal searches for food. A thirsty animal behaves in a way which will lead to satisfying its thirst. More often than not, the behavior of an organism results from a combination of both external and internal stimuli. Internal stimuli, such as hunger, provide the *motivation* for the action taken when food is actually seen or smelled.

Innate vs. learned behavior

In studying behavior, it is worthwhile to try to distinguish between innate and learned forms of behavior. The former are responses whose nature is, in large measure, determined by inherited receptor-conductor-effector patterns. They are usually quite inflexible, a given stimulus giving rise to a given response. A salamander raised away from water until long after its cousins begin swimming successfully will swim every bit as well as they the very first time it is placed in water. Clearly this rather elaborate response is "built in" the species and not something that must be acquired by practice. Learned behavior, on the other hand, is behavior which has become more or less permanently altered as a result of the experience of the individual organism. Only by diligent practice can one learn to play baseball well. Between these extremes, there are patterns of behavior that partake of both innate and learned components. Hailman has demonstrated that the pecking behavior of newly hatched sea gulls, long thought to be innate, is nonetheless strongly influenced by the chick's early experience. (You may read of his findings in his article listed at the end of this chapter.)

INNATE BEHAVIOR

29.2 BEHAVIOR IN PLANTS

Behavior in plants seems to be innate. All the members of any one species respond in the same inflexible way to a given stimulus. There is nothing voluntary about any plant response.

Plants do not possess a nervous system and, as we saw in Chapter 24, plant behavior is restricted to growth movements and turgor movements.

Tropisms

Movements whose direction is determined by the direction from which the stimulus strikes the plant are called **tropisms.** The bending of the oat coleoptile toward a source of light is a positive phototropism (see Section



Fig. 29.1 Chemotaxis in *E. coli.* The bacteria have congregated at the opening of a capillary tube filled with a weak solution of an amino acid. (Courtesy of Dr. Julius Adler from J. Adler, *Science,* **166,** 1588–1597, December 26, 1969.)

24.2). Generalized movements, that is, movements which are not oriented in a particular direction, are called nastic movements or nasties. The opening of tulip flowers on warm days is a thermonasty.

29.3 TAXES

Some organisms respond to a stimulus by automatically moving directly toward or away from or at some definite angle to it. These responses are called **taxes.** They are similar to the tropisms of plants except that actual locomotion of the entire organism is involved. Even as "simple" an organism as *E. coli* displays this behavior. When a capillary tube containing a substance such as glucose is placed in a medium containing *E. coli,* the bacteria alter their pattern of locomotion in such a way that they congregate near the source of the substance (Fig. 29.1). This response, called a **chemotaxis,** does not depend on the organism's ability to metabolize the substance, although presumably that is the value of the response under normal conditions. *E. coli* responds strongly to a number of organic molecules besides glucose, including galactose and the amino acids serine and aspartic acid.

Chemotaxis in E. coli

Phototaxis

Photosynthetic microorganisms often display **phototaxis.** Both the green alga *Chlamydomonas* (Fig. 29.2) and *Euglena* swim directly toward light of moderate intensity. However, as the intensity of light increases, a point is reached where they abruptly reverse direction and swim away from the light (a negative phototaxis).

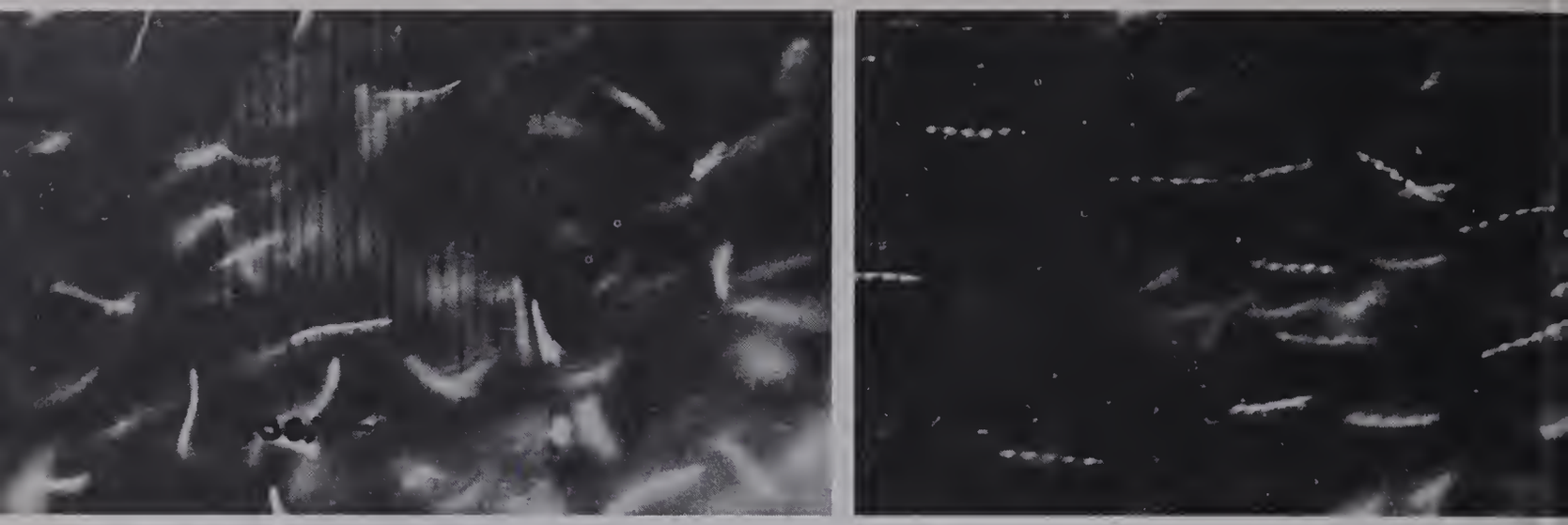


Fig. 29.2 Phototaxis in *Chlamydomonas.* Left: randomly-oriented tracks formed during 1/3 sec by these unicellular algae swimming about in red light (to which they are insensitive). Right: upon adding a beam of blue-green light from one side, the tracks become oriented in its direction. (Courtesy of Dr. Mary Ella Feinleib, Tufts University.)

29.4 THE SIMPLE REFLEX

Probably the simplest innate response among animals having a nervous system is the simple reflex. This is an automatic response of part of the body to a specific stimulus. The response is *inborn;* that is, its nature is determined by the inheritance, not the experiences, of the organism. Two main types of simple reflexes occur in humans, and both seem to have a definite function in the body. The sudden withdrawal of one's hand from a painful stimulus, such as a hot object, involves a *flexion* reflex (see Section 27.4). This response is definitely self-protective. The second category of simple reflexes is the *stretch* reflexes. The familar knee-jerk reflex is one of these. You have undoubtedly had your doctor tap you just below the knee cap with a rubber-headed hammer. Your response, a sudden kick with the lower leg, is an example of a stretch reflex. You may well wonder what value this reflex has under normal circumstances. Actually, it is important in maintaining balance and posture. If a standing person begins to lose his balance, proprioceptors in the tendons and muscles of the leg initiate nerve impulses which travel through the reflex arc and produce corrective muscular contraction in the legs. Remember that even simple reflexes such as this one involve the coordinated contraction of certain muscles and the inhibition of others.

Flexion reflex

Stretch reflex

29.5 INSTINCTS

Instincts are complex behavior patterns which, like simple reflexes, are inborn, rather inflexible, and of value in adapting the animal to its environment. They differ from simple reflexes in their degree of complexity. The

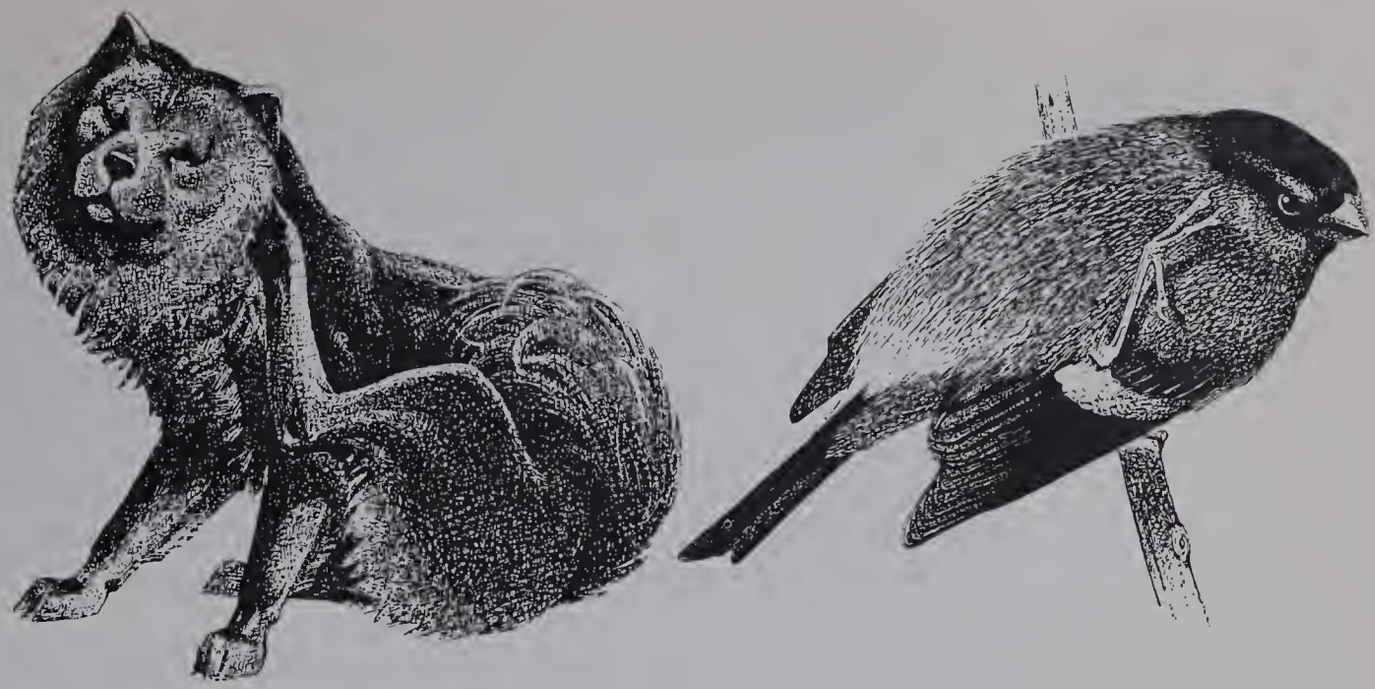


Fig. 29.3 The scratching behavior of a dog and a European bullfinch is part of their genetic heritage and is not changed by training. The widespread habit of scratching with a hindlimb crossed over a forelimb is common to most birds, reptiles, and mammals. (Courtesy of Rudolf Freund and *Scientific American.*)

entire body participates in instinctive behavior, and an elaborate series of actions may be involved. Instinctive behavior is probably the most important type of behavior in insects. Fishes, reptiles, and birds also depend to a large degree on inborn, instinctive patterns of behavior.

Hereditary basis of instinctive behavior

The spider's building of a web is an example of instinctive behavior. A long series of complex actions are required to spin the web, but these, and thus the final shape of the web, are entirely dependent upon instinct. A spider spins a web which is characteristic of its species even though never before exposed to that particular pattern. Nest building in birds is also an example of instinctive behavior. Even when it has never been allowed to see another member of its species or any nest, the weaverbird builds a nest characteristic of the species. Instincts, then, are inherited just as the structure of tissues and organs is inherited (Fig. 29.3).

This fact has been beautifully demonstrated by breeding experiments between closely related species with differing instinctive behavior patterns. The African peach-faced lovebird carries nesting materials to the nesting site by tucking them in its feathers. The closely related Fischer's lovebird uses its bill to transport nesting materials. When these two species are mated, the offspring are successful at transporting materials in the bill only. Nevertheless, they invariably go through the motions of trying to tuck the materials in their feathers first.

Inflexibility of instinctive behavior

The relative inflexibility of instinctive behavior has been demonstrated with many organisms. The tropical army ant (genus *Eciton*) gets its

name from the foraging marches that the entire colony makes over the jungle floor. While the motions of the colony suggest elaborate military maneuvers, in reality they arise from the simple interplay of three factors: (1) the stimulus to move, (2) the tendency of individual ants to remain close to one another following the pheromone laid down by those in front, and (3) the presence of obstacles or food in the line of march. On occasion, these factors become arranged in such a way that the behavior of the ants is truly revealed as blind and instinctive rather than the result of conscious, warlike motives. On a flat surface, such as a paved road, the lead ants begin to move away from the swarm, but their conflicting tendency to stay with the group results in their walking a circular path. The chemical trail laid down is followed by the others and soon the entire swarm is marching around and around in a circle (Fig. 29.4). Unless some obstacle interrupts the path established, the ants will march themselves to their own destruction.

In most circumstances, instinctive behavior promotes the survival of the species. To the human observer, the complexity and usefulness of the behavior suggests the presence of reason and foresight in the animal. It is only when unusual circumstances arise that the true, inflexible, unthinking nature of instinctive behavior is revealed.

The carrying out of instincts often depends upon the conditions in the internal environment of the organism. In many vertebrates, courtship and mating behavior will not occur unless sex hormones are present in the bloodstream. The target organ, in at least some cases, appears to be a small region of the hypothalamus. When stimulated by the presence of sex hormones in its blood supply, the hypothalamus initiates the activities

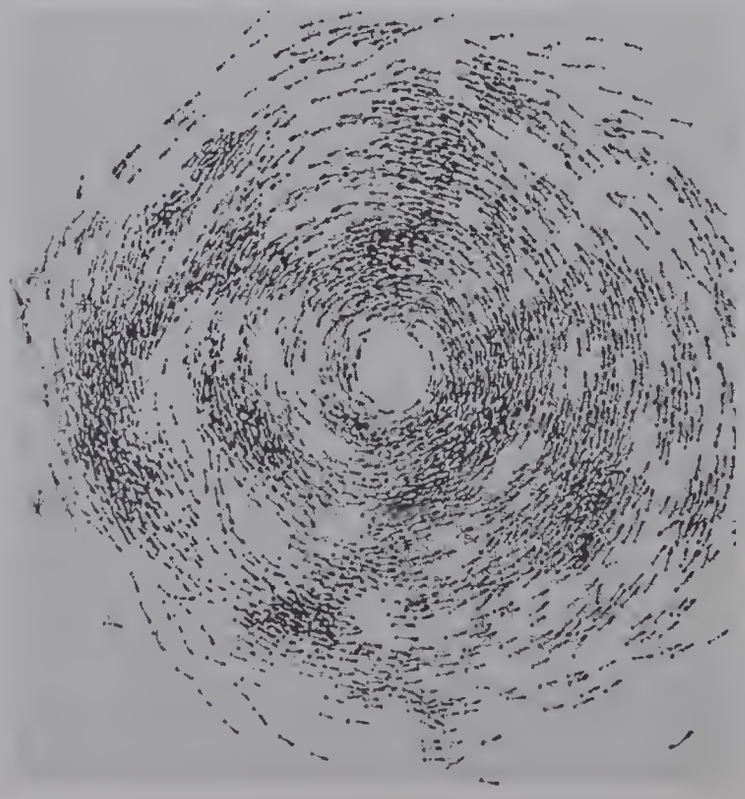


Fig. 29.4 The inflexibility of instinctive behavior. The circular column of army ants *(Eciton)* formed spontaneously and lasted for over 30 hrs. (Courtesy of Dr. T. C. Schneirla, American Museum of Natural History, November, 1944.)

leading to mating. The level of sex hormones in the blood is, in turn, regulated by the activity of the anterior lobe of the pituitary gland (see Section 25.9). Figure 29.5 outlines the interactions that lead an animal, such as the rabbit, to seek a sexual partner and mate with it.

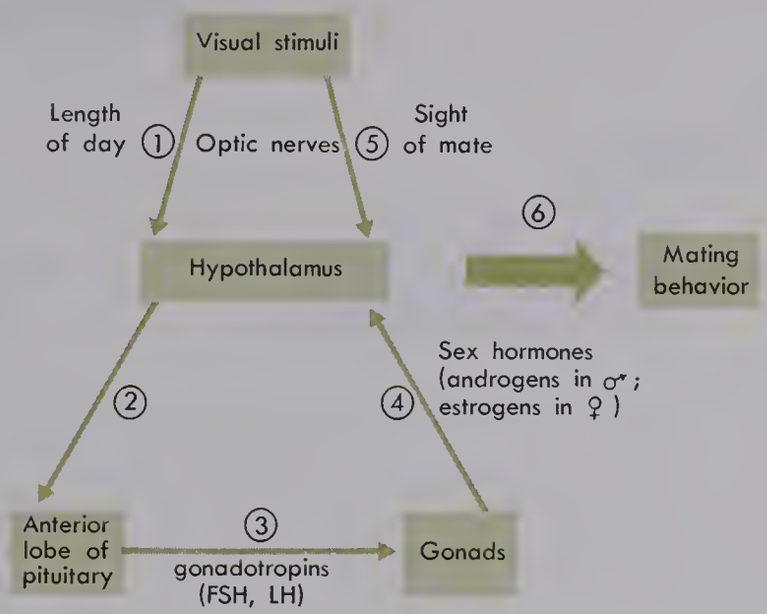


Fig. 29.5 Interaction of external and internal stimuli leading to mating behavior in the rabbit.

29.6 RELEASERS OF INSTINCTIVE BEHAVIOR

Once the body is prepared internally for certain types of instinctive behavior, an external stimulus is needed to initiate the response. N. Tinbergen and his students at Oxford, England, have found that this external stimulus need not necessarily be appropriate to be effective. During the breeding season, the female three-spined stickleback (Fig. 29.6) normally follows the red-bellied male to the nest that he has prepared and lays eggs in it. She will, however, follow almost any small red object presented to her. Once within the nest, the presence of neither male nor red object is necessary any longer. Any object touching her near the base of the tail will cause her to liberate her eggs. It is as though the stickleback were primed internally for each item of behavior and needed only one specific signal to release the behavior pattern. For this reason, signals which trigger instinctive acts are called **releasers.** Once a given response has been released, it usually runs to completion even though the effective stimulus is immediately removed. One or two prods at the base of the tail will release the entire sequence of muscular actions involved in the liberation of the eggs.

The stickleback

Star orientation in warblers

Perhaps the most remarkable releasers of instinctive behavior yet discovered are the stars. The experiments of the German ornithologist E. G. F. Sauer indicate that European warblers, migrating at night, navigate by means of them. This is particularly remarkable in view of the fact that in the fall the young birds fly to their winter home in Africa independently of their parents. With the patient help of his wife, Sauer has even raised young warblers entirely apart from other members of their kind. (This is

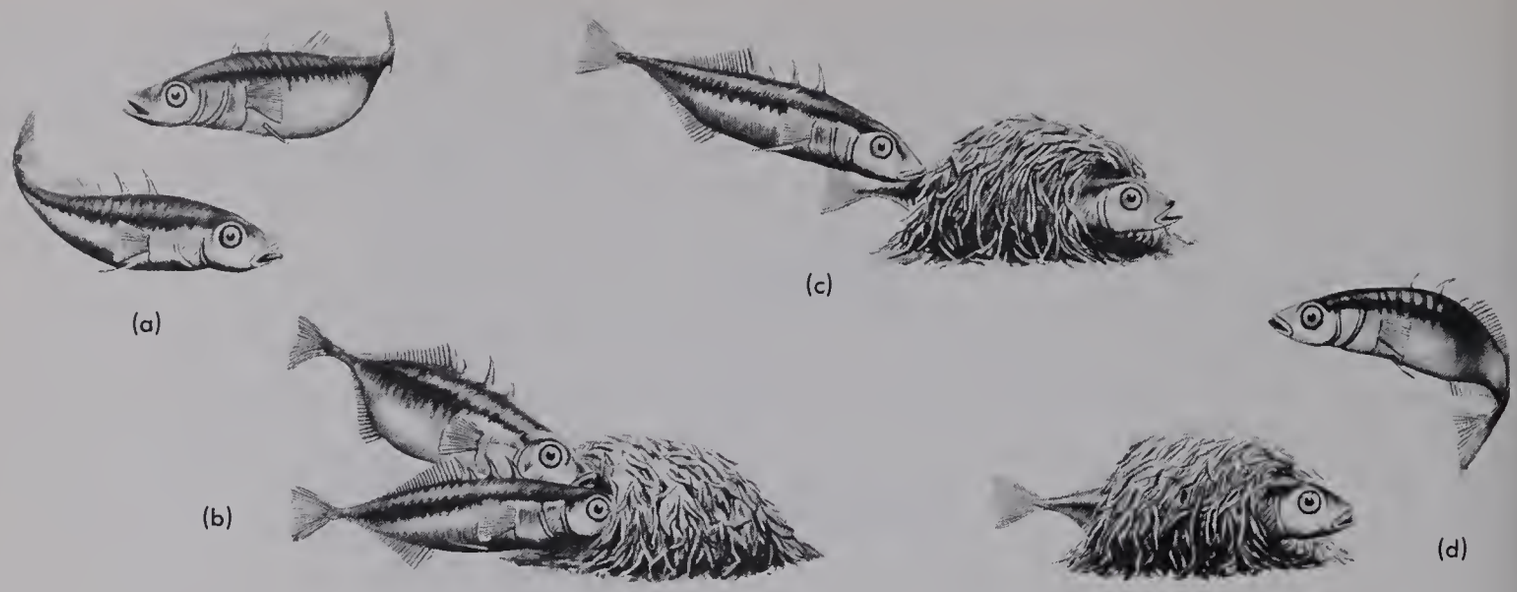


Fig. 29.6 Courtship behavior of sticklebacks. Male leads female toward nest (a), guides her into it (b), then prods the base of her tail (c). After the female lays her eggs, the male drives her from the nest, enters it himself (d), and fertilizes the eggs. (Biological Sciences Curriculum Study, *Biological Sciences: Molecules to Man,* Houghton Mifflin Co., 1963.)

no small task. Warblers eat insects only and a young bird will consume dozens each day.) When fall comes, these young hand-reared birds become restless. Presented with a view of the "stars" inside a planetarium, they orient themselves toward the southeasterly course they would ordinarily follow. Although the constellations are in apparent motion throughout the night, the young birds are able to compensate for this and continue to maintain their proper orientation.

29.7 RHYTHMIC BEHAVIOR AND BIOLOGICAL "CLOCKS"

The migratory "urge" of birds in the fall is one of many examples of behavior that is repeated at definite intervals. Such behavior is described as rhythmic or periodic. The cycles of rhythmic behavior may be as short as two hours or as long as a year. The common laboratory rat provides an example of a very short cycle. Even when food is available at all times, it generally feeds at two-hour intervals.

Circadian rhythms

A great many animals alter their behavior on a daily basis. Nocturnal animals, for example, become active once every 24 hours. Fruit flies hatch in greatest numbers just at dawn. You might well claim that such periodic behavior is simply a response to the daily changes of light and darkness. This is not the complete answer, though. Even when the animals are kept under constant environmental conditions (e.g. under continuous illumination in the laboratory), many of these rhythms continue. They may, however, tend to drift one way or the other. In other words, the cycles may occur every 23 or every 25 hours instead of exactly at 24-hour intervals.

For this reason, such rhythms are called *circadian,* from the Latin words *circa* (about) and *dies* (day). Under natural conditions of alternating night and day, most of these rhythms remain correctly adjusted to a 24-hour cycle.

Cycles of approximately two and four weeks are known. In Section 21.2, we discussed how the male and female California grunions (fishes) come on the beaches to spawn at the time of the full moon and new moon, that is, at intervals of about two weeks. This behavior clearly seems to be triggered by the phase of the moon and/or the height of the tides (which reach a maximum at full moon and new moon). The human menstrual cycle is a 28–30 day cycle which is now, at least, independent of the phase of the moon (and now more physiological than behavioral).

Annual cycles are usually governed by photoperiod

The reproductory behavior of the California grunion does not occur throughout the year but only in the spring. Thus its two-week cycle is superimposed on an annual cycle. Other animal activities also occur on a yearly basis. A few examples are the preparations for hibernation carried out by many mammals, the migration of birds, and the onset and termination of diapause in insects. As we have seen in the case of insect diapause (see Section 25.2), these responses generally depend on the only reliable indicator of the time of year: the relative length of day and night. In other words, most of these activities are regulated by changes in the photoperiod.

The ability to respond to photoperiod requires that the organism have some mechanism for measuring the hours of daylight or the hours of darkness. (Some organisms seem to measure one, some the other.) In other words, these organisms must have some sort of biological "clock." Although the exact nature of the clock mechanism is still unknown, various physiological activities have been found to fluctuate on a daily basis within the organs, tissues, and even the individual cells of organisms with circadian rhythms. Perhaps it is these internal circadian rhythms, which can maintain their periodicity independent of fluctuations in the external environment, that provide the clockwork by which photoperiod can be measured.

29.8 THE LIFE HISTORY OF THE HONEYBEE

Probably no group of animals has developed such a varied repertory of instinctive behavior as the colonial insects—the ants, termites, and honeybees. There is not space in a book of this sort to describe the incredibly diverse and elaborate kinds of behavior which all these animals exhibit, but we shall examine some of the behavior displayed by the domestic honeybee, *Apis mellifera.* (If you are interested in pursuing further the behavior of other colonial insects, reading one or two of the pertinent references listed at the end of this chapter will be well worth your effort.)

The honeybee castes

The life of the honeybee colony revolves around the activities of its single **queen** (Fig. 29.7). During the spring and summer months, she spends most of her time laying eggs in the wax cells of the honeycomb. The queen

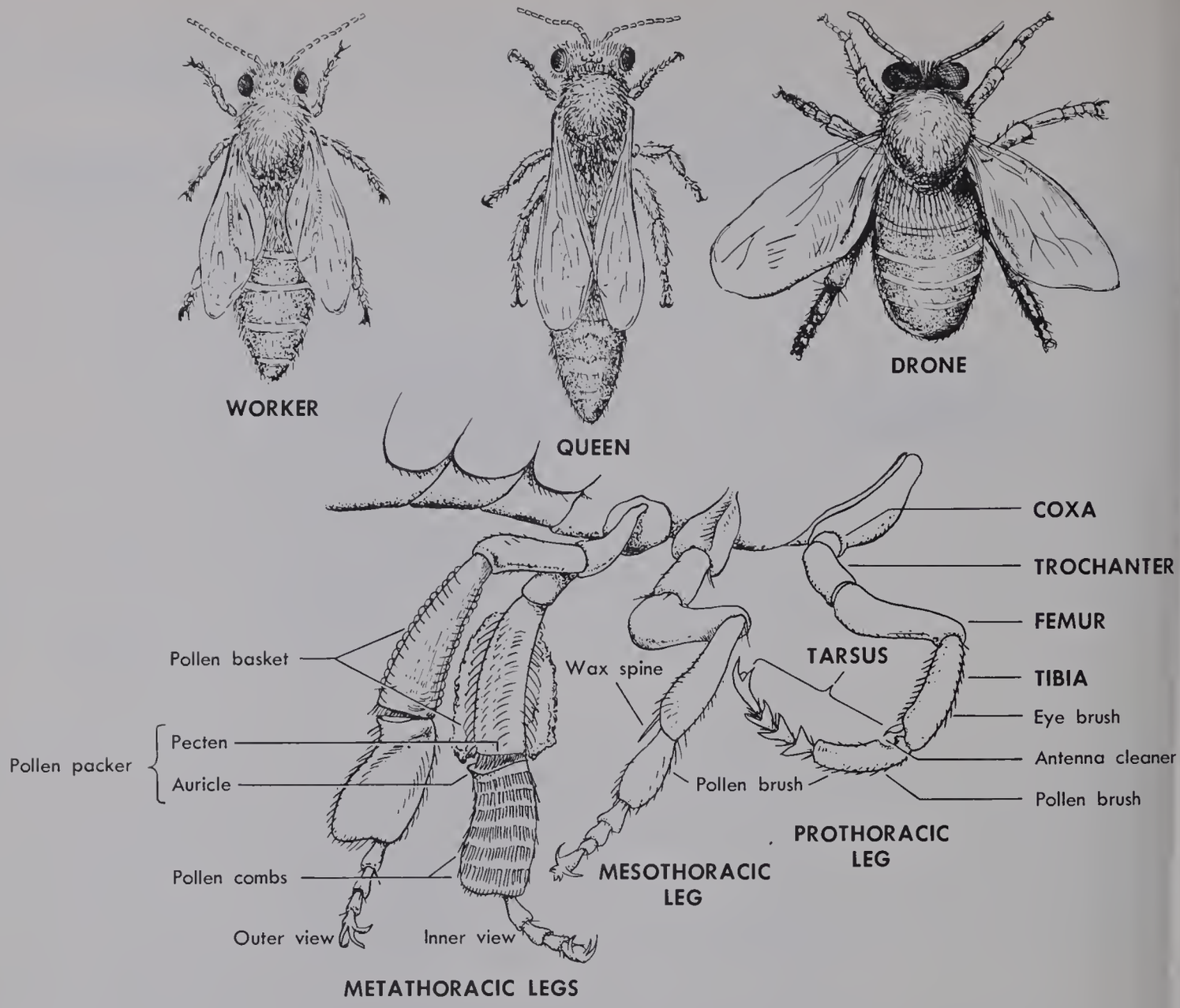


Fig. 29.7 Honeybee anatomy. The three body forms found in the colony are at the top. The legs of the worker bee are shown beneath.

fertilizes most of these eggs, just before they are deposited in the cells, by releasing sperm from storage sacs (sperm receptacles), which were filled at the time of her mating flights. These eggs hatch into larvae (the grubs) after three days. The larvae are carefully tended and fed by **worker** bees for six days. At the end of this time, the workers cap the cells with wax, and the larvae undergo metamorphosis. Three weeks after the egg is laid, a new worker bee emerges. She is a female but does not have functional sex organs.

Early in the spring the queen bee deposits a fertilized egg in each of several special cells, the queen cells (Fig. 29.8). These develop into fertile females, one of which will become the future queen of the hive. The different fate of these eggs is probably accounted for by differences in the diet supplied to the larvae. Young larvae are fed a protein-rich secretion from the salivary glands of adult bees. The composition of the secretion

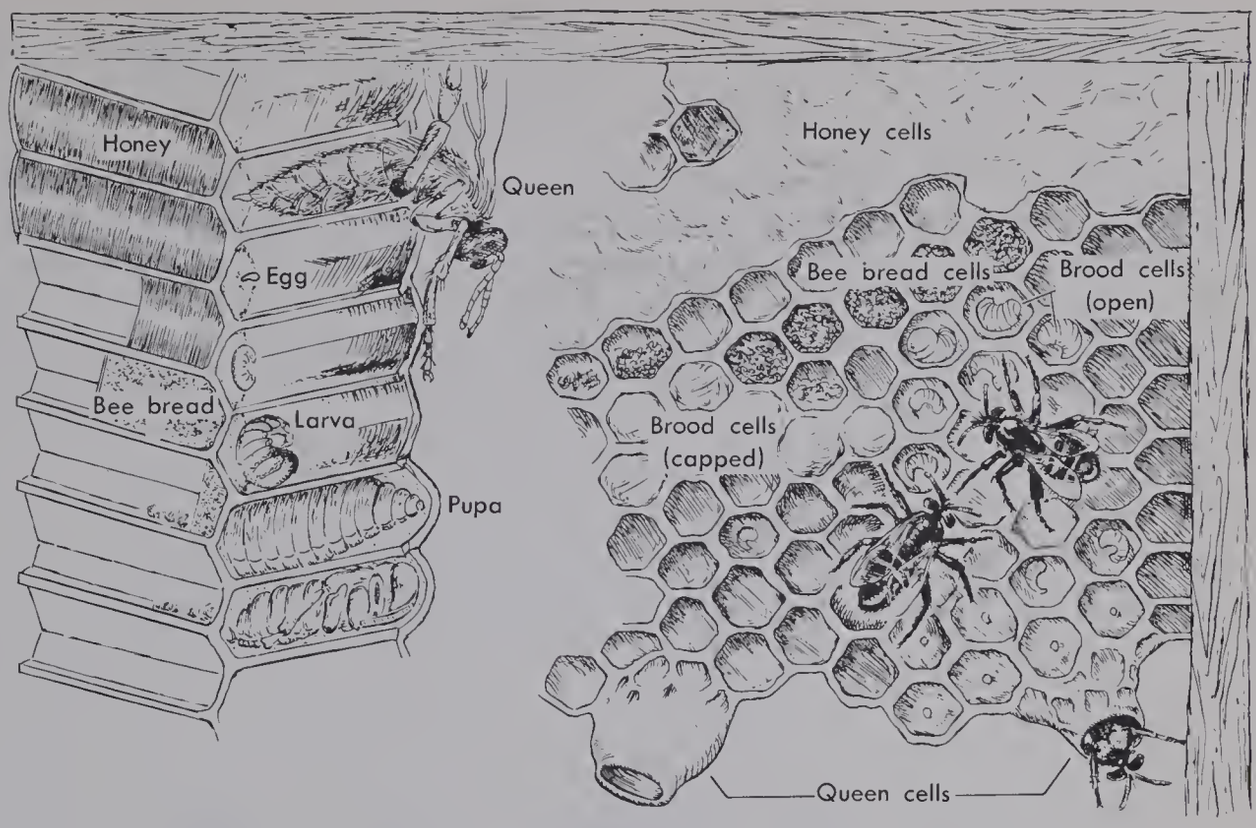


Fig. 29.8 Honeybee comb as seen face on (right) and in section (left). The cells are constructed by the workers out of wax secreted by glands in their abdomen.

fed to the larvae destined to become workers differs from that fed to larvae in the queen cells. The latter material is called "royal jelly."

The queen bee can also lay unfertilized eggs. These hatch and develop into male bees, the **drones.**

The active, normal beehive thus contains one queen, a few hundred drones and many thousands of workers. Before a new queen emerges, the old queen leaves the hive, taking a substantial proportion of the workers with her. This is the phenomenon of swarming. After a few days, the new queen leaves the hive, too, but just long enough to mate (while flying high in the air) with several drone bees. Then she returns to the hive to begin her egg-laying duties. Other developing queens are killed unless the hive is so crowded that additional swarms are appropriate.

The appearance of several distinct body types in a species is called **polymorphism.** In the case of the honeybees, the different forms (worker, drone, and queen) are each adapted to carry out specific functions.

29.9 THE WORK OF THE HIVE

From the moment a worker bee emerges from her cell, her work for the colony begins. For the first three weeks of her life, she stays within or close to the hive. Her duties during this period are undertaken in a definite sequence.

Phases of work within the hive

Days 1–3. This period is spent in cleaning cells for reuse. The worker feeds upon a pollen-honey mixture, bee bread, and her salivary glands become greatly enlarged.

Days 4–9. This period is devoted to nursing the grubs. At first the worker feeds them the protein-rich secretion of her salivary glands. After her glands shrink to normal size, she feeds them bee bread instead.

Days 10–16. The wax glands on the ventral side of the last four abdominal segments begin to secrete beeswax. The worker uses her wax spine (Fig. 29.7) to remove the plates of wax and her mandibles to mold them into new cells for the comb.

Days 17–19. During this period, the workers receive the nectar which foraging bees bring back to the hive, convert it into honey, and store it in cells. Honey production involves evaporating water from the nectar and digesting the sucrose into glucose and fructose. If the hive gets too hot, the workers receive *water* from the foragers and spread it on the surface of the combs. By fanning the water with their wings, they hasten its evaporation, thus cooling the hive. During this period, the workers also remove any debris (e.g. dead workers) that may collect in the hive.

Day 20. This is spent patrolling in front of the hive and attacking and stinging any intruders. A worker can often sting another insect and survive, but her sting usually cannot be removed from the elastic skin of vertebrates. It is pulled from her body, inflicting fatal damage to her abdominal organs.

Two additional points should be noted about the work of the hive. The sequence just outlined is somewhat flexible. Worker bees spend a good deal of time simply patrolling the hive, and they will shift their activities to remedy special needs that they discover. Although bees are proverbially busy, they actually spend only about 40% of their time in the activities described. The remainder is spent simply standing around in the hive. They work hard enough, however, so that their life span as an adult rarely exceeds six weeks in the summer. Workers hatching in the fall have virtually nothing to do, and most of them remain alive throughout the entire winter.

Foraging

After three weeks in or near the hive, the summer workers take to the field as **foragers** of nectar and pollen. The nectar is collected in a special chamber (the honey stomach—Fig. 7.6) of the digestive tract and brought back to the hive to be converted into honey. The pollen is moistened with nectar and brought back in the pollen baskets of the metathoracic (hind) legs (Fig. 29.7). This material is bee bread. The collection of nectar and pollen are vitally important to the plants as well as to the bees. By accidentally transferring some pollen from one flower to the next, the bees enable cross-pollination of the plants to take place with subsequent sexual reproduction and the development of seeds.

29.10 RECEPTORS, CONDUCTORS, AND EFFECTORS IN THE HONEYBEE

Odor and taste

The intricate, well-coordinated activities of the bees would be impossible without good receptors, conductors, and effectors. The large, multifaceted compound eyes (see Section 26.2) provide a fair amount of pattern discrimination (Fig. 29.9) as well as four-color vision. The odor receptors, which are located on the antennae, enable the bee to discriminate among a wide variety of odors and to detect rather faint ones. Despite many claims to the contrary, though, the bee's ability to detect and discriminate among odors is probably no better developed than man's. The mouth parts of the bee contain taste receptors. The bee seems to distinguish the same four taste sensations that we do: sweet, sour, salty and bitter. The bee's threshold to sour and salty is lower than man's while its threshold to bitter and sweet is higher. The relatively high threshold to sweet is of value to the bee as it prevents her from collecting nectar too dilute to be converted efficiently into honey.

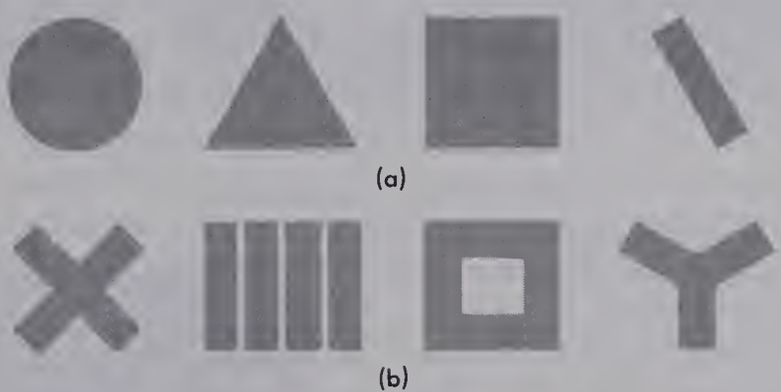


Fig. 29.9 Pattern discrimination in honeybees. Bees cannot distinguish between any of the figures in row (a) or row (b) but can distinguish any figure in row (a) from any in row (b).

The nervous system of the worker bee is well organized to receive information from the sense receptors and to coordinate the action of the muscles controlling legs, wings, and mouth parts.

The legs of the honeybee

The legs of the honeybee are far more than simply structures to walk on. They are highly specialized manipulative organs which enable the bee to carry out many of its tasks.

The anterior legs have soft hairs (the eye brush) on the tibia (Fig. 29.7). As the name implies, the eye brush is used for removing pollen and other debris from the eyes. The pollen brush on the first tarsal segment is made of rather stiff bristles. It is used to brush pollen off the body hairs. The first tarsal segment also contains a notch in which the bee can place its antenna. Then when the bee crooks the leg, a spur on the tibia holds the antenna in the notch while the bee cleans off pollen, etc., in a wiping motion. The entire apparatus is called the antenna cleaner.

The middle, or mesothoracic, legs also contain pollen brushes on the first tarsal segments. In addition, a wax spine sticks down from the tibia. This is used to dig plates of wax out of the wax glands.



Fig. 29.10 Worker honeybee foraging for pollen. Note the full pollen baskets on the metathoracic legs. (Courtesy of W. T. Davidson, National Audubon Society.)



Fig. 29.11 Applying paint to foraging bees so that they can be identified on their return to the observation hive. (Courtesy of Dr. M. Renner.)

Each hind or metathoracic leg contains pollen combs on the inner surface of the first tarsal segment, a pollen packer in the joint between the tibia and the first tarsal segment, and a pollen basket on the outer surface of the tibia. The pollen combs collect pollen from the pollen brushes of the other legs. Then with the stiff spines (the pecten) of the pollen packer of one leg, the bee removes the pollen from the pollen combs of the opposite leg. Straightening of the leg then forces the anvil-shaped auricle up against the pollen caught under the pecten. The pollen is squeezed through the joint and up into the pollen basket on the outside of the tibia.

All these manipulations are carried out in the brief interval it takes the bee to fly from one flower to another. When pollen is plentiful, the amount transported in the pollen baskets is truly impressive (Fig. 29.10).

The mouth parts of the honeybee (look back at Fig. 7.6) are also highly specialized and enable the bee to manipulate the materials essential to the life of the colony. The mandibles are used for kneading wax to make more honeycomb. The maxillae, the labium, and the tongue are modified to form a long proboscis for sucking nectar from the nectaries of flowers.

29.11 COMMUNICATION AMONG HONEYBEES

The colonial way of life places great emphasis upon the home. The hive is the center of activity and, no matter how far afield the workers roam, they almost invariably return to their own hive. Considering that bees may forage several miles from the hive, they must be good navigators to find their way back successfully. Certainly bees have sufficiently good vision to be able to navigate by means of prominent landmarks, and evidence suggests that they often do. It has also been discovered, however, that most foragers do not leave the hive until food has actually been discovered by so-called **scout bees.** As soon as the scouts find food, they return to the hive. Shortly after their return, many foragers leave and fly directly to the food supply. The remarkable thing about this behavior is that the foragers do not follow the scouts back to their discovery. Instead, they fly to the food while the scouts are still within the hive. This implies two things. First, the foraging bees must have been told in some way how to locate the food source. Second, they must have some method of navigating over unfamiliar territory as they follow these instructions.

Scout bees

The discovery of how scout bees communicate with the foragers and how the foragers navigate over unfamiliar territory was made by the German zoologist Karl von Frisch. Throughout his entire professional career, he has patiently studied and experimented with honeybees. Many of the facts of bee life which we have discussed were discovered by him.

By marking scout bees with paint and watching them upon their return to an observation hive (Fig. 29.11), von Frisch discovered that the scouts perform a little dance on the vertical surface of the combs after depositing their load of nectar or pollen. This dance seems to stimulate the foragers and soon they begin to leave the hive and fly to the food source.

The tailwagging dance

Unless the food source is quite close (less than 75 yards) to the hive, the scout bees dance a so-called tailwagging dance (Fig. 29.12). It did not take von Frisch long to realize that the speed with which the scout bees perform this maneuver is related to the distance of the food from the hive (Fig. 29.13); the scouts even compensate for wind speed. However, the knowledge that food is present three miles from the hive is not very useful when you consider the long circumference included. But von Frisch also noted that the direction of the tailwagging portion of the dance varies with the direction of the food source from the hive and with the time of day. At a given time of day, the direction of the dance changes with varying locations of food. With a fixed source of food, the direction of the dance changes by the same angle as the sun during its passage through the sky.



Fig. 29.12 Left: the round dance of the bee is used when food is found close to the hive. Right: the tail-wagging dance is used when food is found more than 100 yds from the hive. The speed of the dance indicates just how far away the food is, and the direction of the straight portion indicates in what direction.

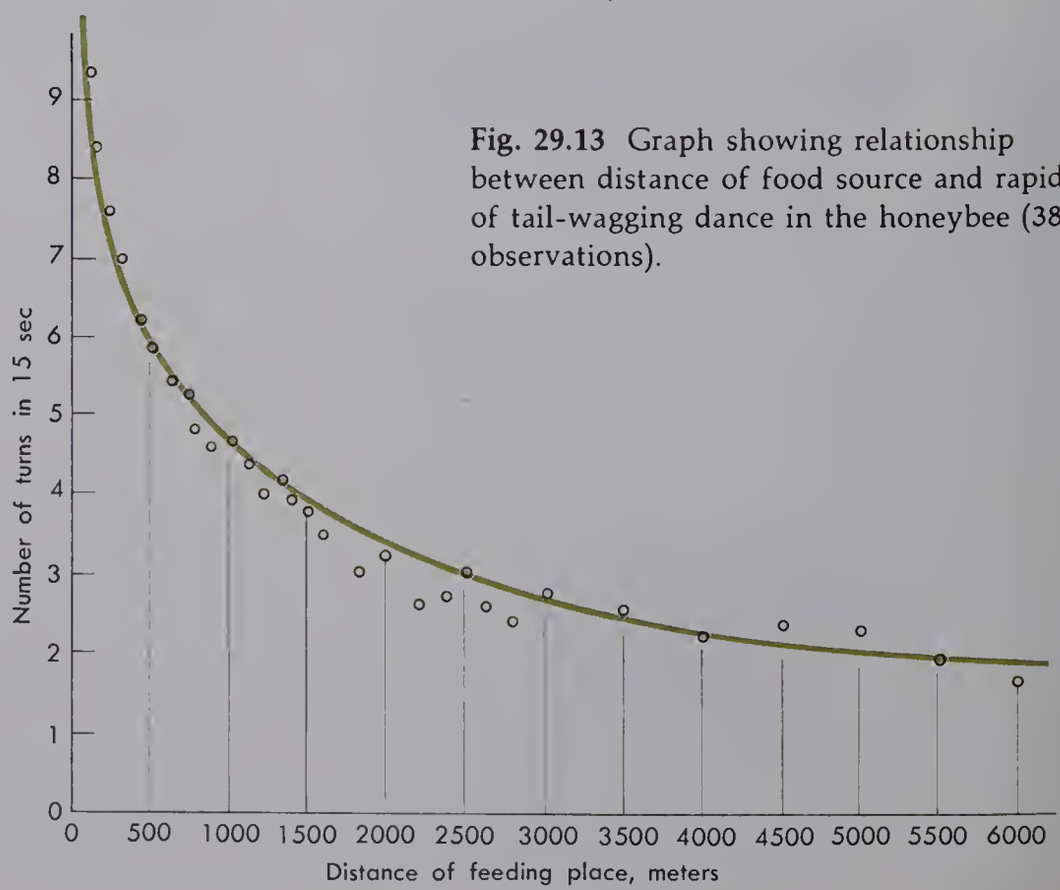


Fig. 29.13 Graph showing relationship between distance of food source and rapidity of tail-wagging dance in the honeybee (3885 observations).

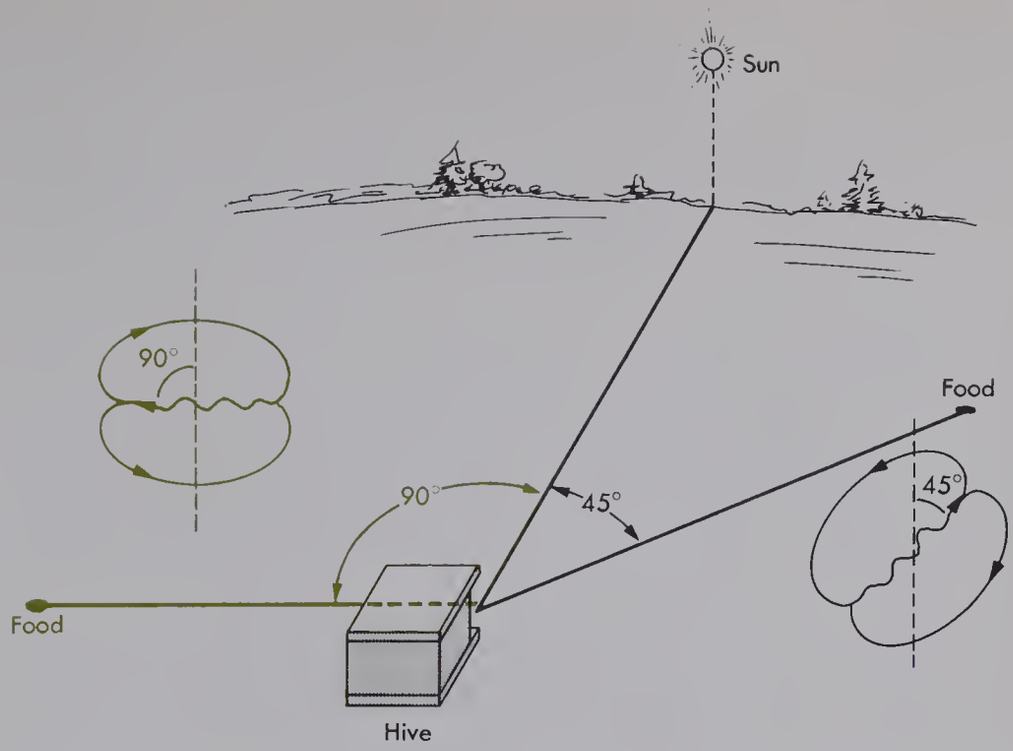


Fig. 29.14 Relationship between the angle of the dance on the vertical comb and the bearing of the sun with respect to the location of food. When the food and the sun are in the same direction, the straight portion of the dance is directed upward. When the food is at some angle to the right (black) or left (color) of the sun, the bee orients the straight portion of its dance at the same angle to the right or left of the vertical.

This suggests that the scout bees indicate the direction of the food source in relation to the direction of the sun. The sun is not visible in normal hives, however, and the bees dance on the vertical surface of the combs. How, then, do they translate flight angles in the darkened hive? When the food source is in the same direction as the sun, they orient the straight portion of the wagging dance up and down with their heads pointing up. It is as though they translate a positive phototaxis as a negative geotaxis. If the food source is at some angle to the right or left of the sun, the scouts dance at the same angle to the right or left of the vertical (Fig. 29.14).

The foraging bees cluster around the dancing scouts and presumably learn the direction and distance of the food in this way. If the food source is scented, the foragers also learn what odor to search for. Thus the language of the bees enables one bee to tell the others: (1) that food is available, (2) the direction to the food, (3) the distance to the food, and (4) the odor of the food.

The time sense of honeybees

If the dancing bees are confined to the hive for a long period of time, they shift the direction of their dance as the direction of the sun shifts. Remember, though, that they cannot observe the apparent motion of the sun while within the hive. This suggests that the bees are able to make the necessary corrections because they are "aware" of the passage of time. This fact has long been known to people who like to have tea, toast, and jam

in their gardens at a fixed time every day. Within minutes of the appointed hour, foraging bees arrive in large numbers for their share of the jam. Here, then, is another example of a biological "clock." Its rate of ticking seems to be related to the bee's rate of metabolism. If a group of normally punctual bees are chilled (to lower their rate of metabolism) or exposed to anesthetizing concentrations of carbon dioxide, their next arrival at the tea table will be correspondingly delayed (Fig. 29.15).

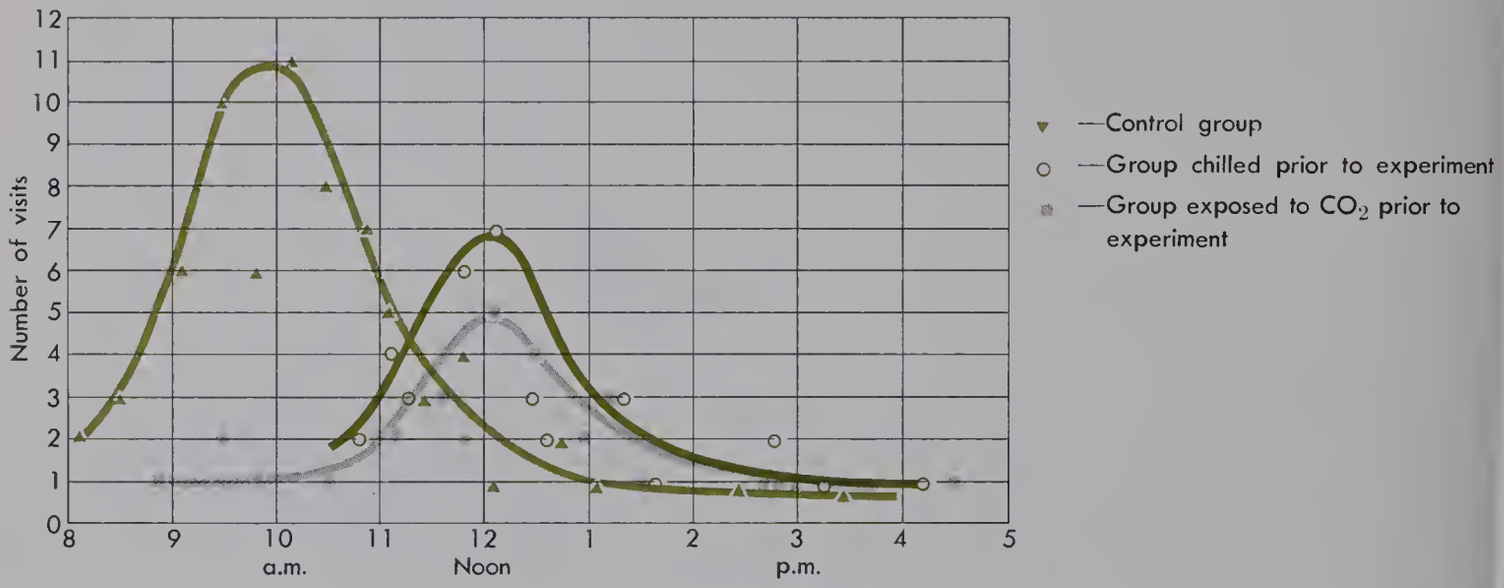


Fig. 29.15 Effect of chilling and exposure to CO_2 on the time sense of honeybees. Both chilling and CO_2 slow the metabolic rate of these animals. Each group had been fed between 9 and 11 a.m. for 4 days prior to the experiments.

These discoveries made by von Frisch are deceptively easy to describe. Like most scientific discoveries, they are the fruit of years of patient work, cleverly designed experiments, and carefully interpreted results. For a better idea of the actual process of scientific discovery, you should read von Frisch's own account (listed at the end of this chapter) of the long series of experiments which led him to these, and many other, discoveries concerning the behavior of bees.

Learning in honeybees

When one first learns about the elaborate behavior of honeybees, he is apt to credit these little creatures with a high degree of intelligence, foresight, etc. Actually, this would be a mistake, since most of their behavior is instinctive and thus relatively inflexible. Within certain narrow limits, however, they can alter their behavior. When behavior is more or less permanently modified as a result of the experiences of the organism, we say that learning has occurred. The bee's association of food with a certain location, odor, or time of day is certainly an example of learning.

LEARNED BEHAVIOR

Learned behavior is behavior that is more or less permanently acquired or modified as a result of the experiences of the individual.

29.12 HABITUATION

Almost all animals are able to learn not to respond to repeated stimuli which have proven to be harmless. This phenomenon is known as habituation and is an example of true learning. If you make an unusual noise in the presence of the family dog, it will respond—usually by turning its head towards the source of the sound. If, however, the stimulus is given repeatedly and nothing either pleasant or unpleasant happens to the dog, it will finally cease to respond. That this is a case of true learning rather than simply the result of the adaptation of sense receptors is indicated by the fact that the response is long-lasting. When fully habituated, the animal will not respond to the stimulus even though weeks or months have elapsed since it was last presented.

29.13 IMPRINTING

Imprinting can occur only during a critical, usually brief, period

One of the most narrowly specialized, clear-cut examples of learning is imprinting. If newly hatched geese (goslings) are exposed to a moving object of reasonable size emitting reasonable sounds, they will begin following it just as they would normally follow their mother. This is called imprinting. The time of exposure is quite critical. A few days after birth imprinting does not take place. Prior to this time, though, the results are quite remarkable. The gosling imprinted to a moving box or clucking man will, forsaking all others, try to follow this object for the rest of its life. In fact, when the gosling reaches sexual maturity, it will make the imprinted object, rather than a member of its own species, the goal of its sexual drive. Much of our knowledge of imprinting has been gained from the patient research of Konrad Lorenz (Fig. 29.16).

29.14 THE CONDITIONED RESPONSE

Perhaps the simplest form of learned behavior is the *conditioned response.* Basically, this is a response which, as a result of experience, comes to be caused by a stimulus different from that which originally triggered it. We owe our understanding of the mechanism of the conditioned response to the research of the Russian physiologist Ivan Pavlov. Pavlov found that the placing of food in a dog's mouth causes it to salivate. This is probably a simple, inborn reflex involving taste buds, sensory neurons, association neurons in the brain, and motor neurons running to the salivary glands. Pavlov found further that if he rang a bell every time he introduced meat into the dog's mouth, the dog eventually salivated upon hearing the bell

Pavlov's experiments



Fig. 29.16 Konrad Lorenz with imprinted goslings. In 1973, Lorenz shared a Nobel Prize with N. Tinbergen and Karl von Frisch. (Tom McAvoy—courtesy of LIFE Magazine, © 1955, Time, Inc.)

alone. *This* is the conditioned response. The dog has learned to respond to a substitute stimulus, the *conditioned stimulus.*

We assume that the physiological basis of this conditioned response is the transfer, by association neurons, of nervous activity in the auditory area of the brain to the motor neurons controlling salivation. This process involves the use of new circuits which, we may also assume, is characteristic of all forms of learning. In fact, some psychologists feel that all learned behavior arises from the development of conditioned responses. They feel that the conditioned response is the fundamental unit of even the most complex forms of human behavior. While our knowledge of our own higher mental processes is still far too poor to accept or reject this theory outright, there is no question that humans can be conditioned to some degree.

Experiments on conditioning have taught us a good deal about the learning process in man. Conditioning occurs most rapidly when (1) the unconditioned stimulus and conditioned stimulus are presented together frequently, (2) when there are no distractions, and (3) when a reward of some sort is given for successful performance of the conditioned response. Similarly, as every student knows, learning proceeds most successfully with repetition, lack of distraction, and good motivation.

Use of the conditioned response to determine an animal's sensory capacities

The conditioned response has proved to be an excellent tool for determining the sensory capabilities of other animals. As we have seen from Fig. 26.4, honeybees can be trained to seek food on a piece of blue cardboard. They learn to associate the color blue with the presence of food. This is a conditioned response. By offering other colors to the blue-conditioned bee, we can discover which she confuses with blue and which she does not. In this way, Karl von Frisch determined that honeybees can see only four distinct colors: yellow-green, blue-green, blue-violet, and ultraviolet. The ability of animals to discriminate between similar shapes and musical tones has also been studied by the technique of conditioning.

29.15 INSTRUMENTAL CONDITIONING

Pavlov's dog was restrained in one position and the response that was being conditioned (salivation) was an innate one. But the principles of conditioning can also be used to train animals to perform tasks that are not innate. In this case, the animal is placed in a setting where it can move about and engage in a number of different behavioral activities. The experimenter chooses to reward only one, for example turning to the left. By first rewarding (e.g. with a pellet of food) even the slightest movement to the left and then only more complete turns, a skilled experimenter can, in about two minutes, train a naive pigeon to make a complete turn. A little more work and the pigeon will pace out a figure eight. Such training is known as **instrumental conditioning** or **operant conditioning** (Fig. 29.17).

Operant conditioning

The latter term was coined by the psychologist B. F. Skinner (whose skill with the technique enabled him to train pigeons to play ping-pong and even a toy piano!) because it indicates that through its behavior the animal



Fig. 29.17 Instrumental conditioning. Presented with two spots of light, the pigeon pecks at the brighter and then reaches down to pick up the grain of food that is its reward. (Courtesy of Roy DeCarava and *Scientific American.*)

is operating on its environment, i.e. influencing its situation. It is also called trial-and-error learning because the animal is free to try various responses before finding one that will be rewarded.

Maze learning

Maze problems are a form of instrumental conditioning in which the animal is faced with a sequence of alternatives. All animals with bilaterally symmetrical nervous systems can learn to make some simple, consistent choices when confronted with alternatives. In a *T*-maze (Fig. 29.18) an earthworm or a planarian can eventually learn to take the arm of the maze which leads to a reward (e.g. food, moisture) and/or away from punishment (e.g. sandpaper, electric shock). The development of this response is a slow process, however, and never reaches the point of 100% correct choices. Interestingly enough, the earthworm learns to solve this maze even after its cerebral ganglia have been removed; but once new cerebral ganglia regenerate, the worm forgets its prior learning and must be retrained. Ants and mice are excellent solvers of mazes. It takes some ants as few as 28 trials to solve the maze shown in Fig. 29.19. As for Julia (Fig. 29.20), she is able to solve mazes like the one shown on her *first* attempt most (86%) of the time and sometimes faster than biology students can!

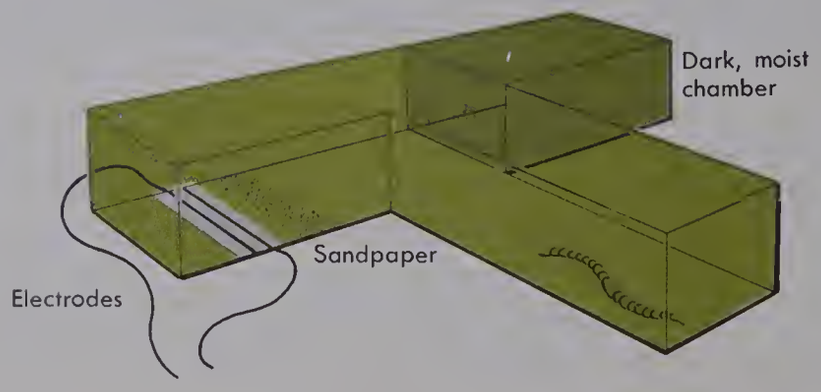


Fig. 29.18 A *T*-maze. Earthworms can learn to make the correct choice 90% of the time.

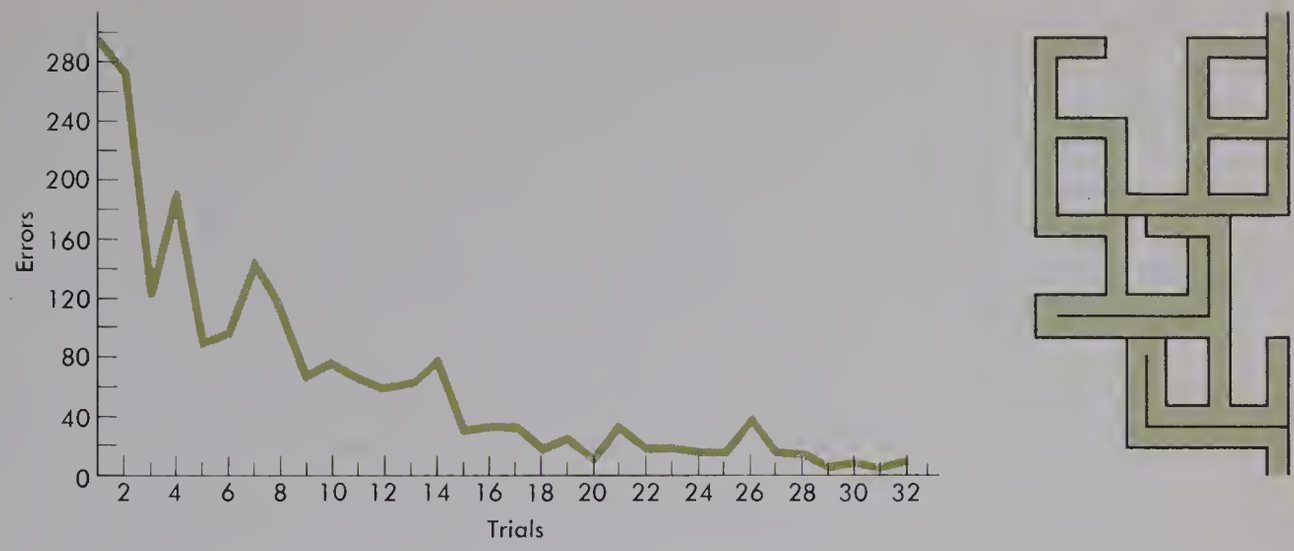


Fig. 29.19 Trial-and-error learning in the ant.

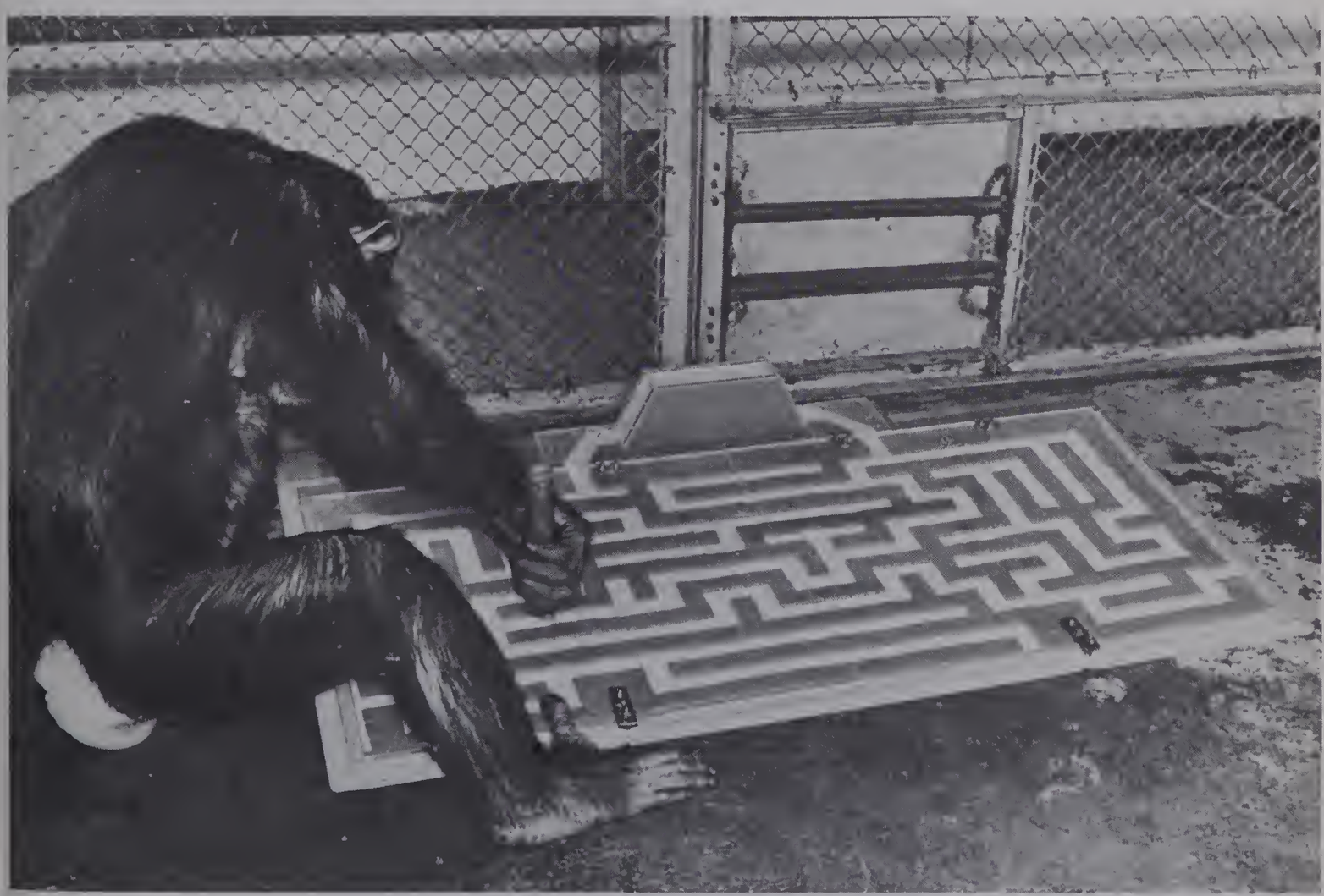


Fig. 29.20 Julia, a chimpanzee, uses a magnet to move an iron ring through the maze. Although biology students *usually* solve such a maze faster than Julia can, this is not always the case. (Courtesy of B. Rensch.)

29.16 MOTIVATION

Motivation involves the satisfaction of drives

Anyone who has tried to train an animal in a procedure, such as running a maze, that requires the animal to participate actively and to make choices knows that it can be a very frustrating procedure. The student psychologist is apt to find that when rat and newly constructed maze are brought together, the rat simply curls up in a corner. The basic problem here is one of motivation. The animal must "want" to participate in the learning process. Among most animals, motivation (or "drive" as it is sometimes called) is connected with its physical needs. A thirsty animal will search for water and a hungry animal will search for food. It is standard practice, for example, to deprive a rat of food for 23 hours prior to using it for instrumental conditioning (when, of course, the reward is food).

The satisfaction of its drives is the motivating force behind the animal's behavior. At times, the internal drive can be very precise. A rat with sugar, or salt, or even thiamine missing from an otherwise adequate diet will seek out food containing the missing substance in preference to that which does not. Perhaps we can go so far as to say that most of the spontaneous behavior of these animals results from an attempt to maintain homeostasis. Many of these drives have their origin in the hypothalamus. In some cases (thirst, for example) the hypothalamus actually detects the deficiency in the ECF. In all cases, the hypothalamus seems to initiate the responses which lead to a reduction of the drive. It may also inhibit some of these responses when the point of satiation is reached.



Fig. 29.21 Curiosity in a monkey. The monkey repeatedly works the puzzle with no other motivation than that created by the task itself. (Courtesy of Myron Davis and *Scientific American.*)

While we can trace a good deal of man's behavior to the desire to satisfy physical needs, not all of it can be explained in such terms. Many of the things we do we seem to do for their own sake. Goats, monkeys, and chimpanzees, too, have been found to engage in problem-solving activity even when no external reward or punishment is involved (Fig. 29.21). The carrying out of the process itself seems to be its own reward.

Delayed gratification

Chimpanzees and men are unusual also in that they will both work for distant goals. Both chimps and men can learn to work for coins even though the coins themselves satisfy no physical need. Of course, the coins can be converted into food, and this expectation provides sufficient motivation for brief periods of work in chimpanzees and a lifetime of work in men.

29.17 CONCEPTS

Most animals solve mazes and other problems by trial and error. As long as sufficient motivation is present, they try each alternative and gradually, through repeated successes and failures, learn to solve the problem (Fig. 29.22). Trial-and-error learning plays a negligible role in man, however. When presented with a problem, he may make one or two random attempts at solving it and then, all of a sudden, he "gets it." We call this response **insight.** (Sometimes it is referred to as the "aha!" reaction.)

The "aha!" response

Responses that occur as a result of insight are quite different from anything we have considered up to now. While they depend upon previously learned material, they are an entirely new response for the individual. Insight involves putting familiar things together in new ways. It is thus a truly creative act. Insight also depends upon the development of concepts or principles. This can be illustrated by a hypothetical experiment (Fig. 29.23). A rat is placed in front of a semicircle of doors, three of which are opened at a time. No matter which particular doors are opened, if the rat enters either the left-hand or right-hand door, it receives a shock. If it enters the middle door, it finds a reward of food. If the rat ever should learn to go immediately to the middle door (even if that door led to a shock on the previous trial), the rat would have learned a **concept.** In this case, the

Insight is creative



Fig. 29.22 A detour problem. A dog finally solves it by trial and error.



Fig. 29.23 If the rat could learn always to go to the middle one of whichever three doors happen to be open, he would have learned a concept. Although a chimpanzee can solve such a problem with ease, the rat cannot.

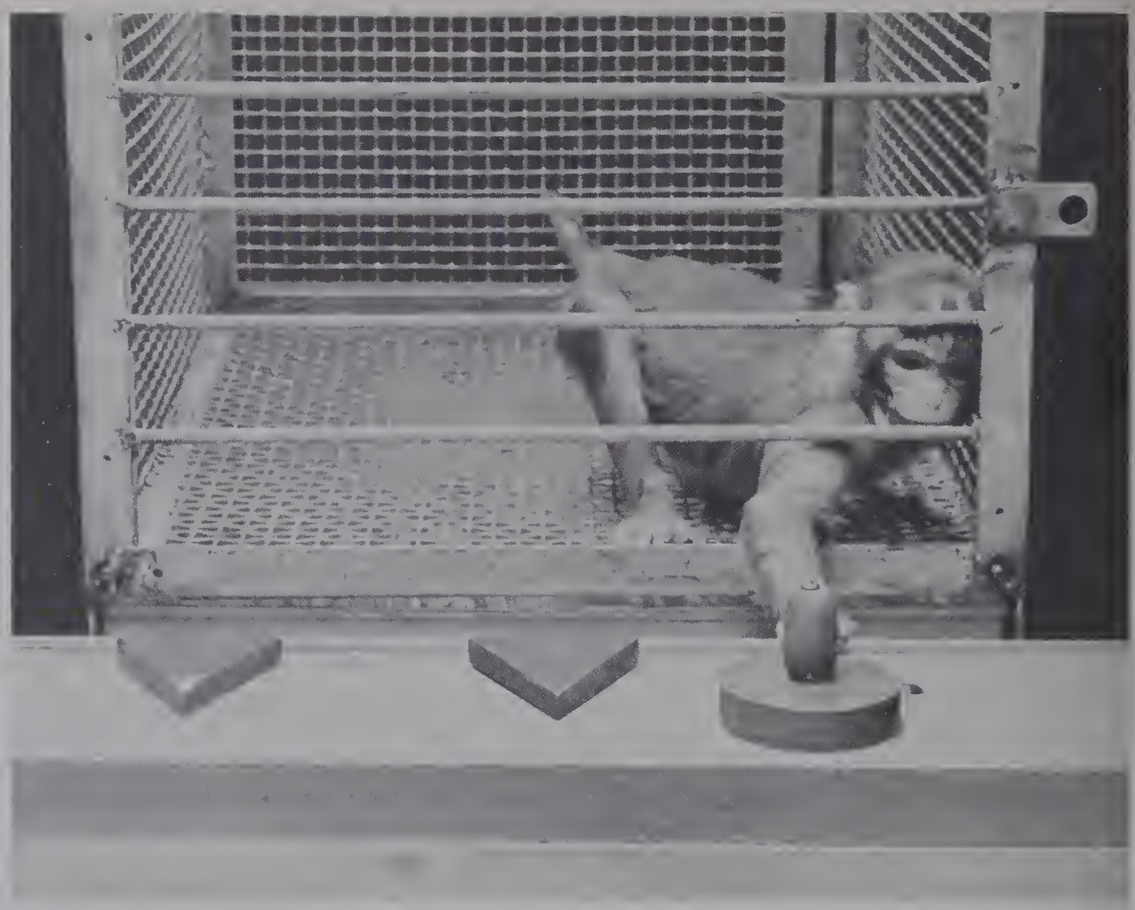


Fig. 29.24 A young rhesus monkey solving an oddity problem. It has learned that food will be found under whatever object is the odd one of the three presented. (Courtesy of H. F. Harlow, University of Wisconsin Primate Laboratory.)

Learning abstractions

concept would be the idea of *middleness.* The rat would not be responding to any concrete, specific stimulus but rather to an idea. From its experience with particular doors it would have developed an idea about doors in general.

Actually, a rat would fail this test. Rats and most other animals have little or no ability to make abstract generalizations about specific things. A man can solve this sort of problem, of course. Chimpanzees are also capable of developing abstract ideas. Monkeys and elephants can solve simple oddity problems (Fig. 29.24).

Problem solving of the type illustrated by our hypothetical rat in front of the semicircle of doors involves some form of reasoning. Chimpanzees, which can solve this problem, are probably reasoning without the aid of a language. Nevertheless, two different, but related, thought processes are involved. One, *inductive* reasoning, involves learning the general principle (middleness) from experience with the specific, concrete situation. The other, *deductive* reasoning, involves applying the general principle to some new specific situation. If the chimp had a language, it might say, "If food is always located behind the middle door, then this time it must be located behind *that* door."

Inductive vs. deductive reasoning

29.18 LANGUAGE

All humans, even in the most primitive societies, possess a highly developed language of some sort. This involves a second level of abstraction. The very idea of a chair or door, like the idea of middleness, is an abstraction. When these ideas are then represented by symbols, a second level of abstraction occurs. There is nothing chairlike about the letters *c h a i r.*

Of course, it would be perfectly possible to create a written language with a unique symbol, such as a sketch, for each concept. This occurred to some degree in the development of language among the Chinese, Japanese, and the Indians of North America. A more flexible arrangement is to use a relatively small number of symbols which can then be assembled in unique ways for each concept. Our written language is made up of 26 letters whose varied arrangement makes our written language possible.

Phonemes

Contrast, if you will, the meanings associated with *r a t* and *t a r.* In a similar way, our spoken language is made up of some three to five dozen (depending on who is doing the counting) distinct sounds or **phonemes.**

For many years it was generally agreed that one feature that distinguished man from all other animals was his use of language. Of course, we now realize that many other animals (e.g. the bees) have signaling mechanisms that possess some of the features of a language. But until recently it was felt that no other creature could, as we do, take a small number of arbitrary symbols and assemble them in unique ways for particular concepts. However, two remarkable series of investigations with chimpanzees suggest that this is not a uniquely human attribute. The husband-and-wife team of Ann and David Premack chose to investigate the language potentialities of the chimpanzee by using metal-backed plastic symbols for words and a magnetized blackboard on which these could be assembled into "sentences." After some half-dozen years of training, their subject—

Sarah's symbolic language

a female chimp named Sarah—had a vocabulary of about 130 words. These included not only nouns (e.g. Sarah, apple, pail) but verbs (is, give, take, wash) and adjectives like red and yellow (the symbols for these were *not* so colored). Sarah also learned to deal with symbols representing such concepts as "same," "different," "not," "if-then," and a question. With these tools, she could clearly grasp some essentials of grammar. Furthermore, she appeared to be "thinking" in her symbolic language. When presented with her word for apple (a blue triangle) she described it as red, round, and with a stem even though no apple was present at the time.

The use of symbols that can be manipulated (instead of spoken words) to explore the language ability of a chimpanzee rests on the evident manual dexterity of chimps and their inability to vocalize. This was also the reason

Washoe's use of the American Sign Language

that the Gardners elected to explore chimp language ability by use of the American Sign Language, which is used by the deaf in North America. While many of the gestures in this language are somewhat representational (Fig. 29.25), many others are not. After 22 months of training, their young female chimp, named Washoe, had learned more than 30 signs. With these she was able to make a variety of requests and to answer simple questions.



Fig. 29.25 Washoe, a young female chimpanzee, gives the sign for "drink" in the American Sign Language of the deaf. (Courtesy of R. Allen Gardner and Beatrice T. Gardner.)

When she thought she was not being observed, she would "talk" to herself or name the objects in a picture book. Often, on the way to the toilet, she made the sign for "hurry."

While the skills displayed by Sarah and Washoe may appear rudimentary, they are every bit as good as those of a two-year-old child. Those who believe that human linguistic ability differs qualitatively, not just quantitatively, from that of a chimpanzee would now seem to be saddled with the burden of proof.

29.19 MEMORY

All learning depends upon memory. If the organism is to modify its behavior from experience, it must be able to remember what its experience has been. Once something is learned, memory is necessary for the learning to be retained.

Dynamic theory of memory

Two basic theories of memory have been proposed. One is that memory is a dynamic process. According to this theory, sensations give rise to nerve impulses, which then circulate indefinitely through the network of neurons in the central nervous system. When one considers the vast network of association neurons in the human cerebrum, such a theory seems

plausible. The delay circuits used to store information in some modern computers operate on the same principle. This dynamic theory of memory is supported by the startling fact that no specific area of the human brain has ever been found essential to the retention of old memories. Whatever defects may occur as the result of damage to one region of the brain or another, loss of memory does not seem to be involved. On the other hand, a dynamic memory must always be in action. If all nerve impulses in the brain ceased, even for an instant, then this type of memory would be lost. However, when maze-trained rats have been carefully chilled to the point where no electrical activity in the brain can be detected and then rewarmed, they remember their prior training.

This fact supports the second theory of memory which is that each remembered sensation results in some permanent, physical alteration in the brain. Perhaps a change in the resistance of certain synapses occurs, although the fact that memory does not seem to be localized anywhere in the brain argues against this view. Recently, several biologists have suggested that our memories may be stored in a chemical code within the brain. Some look to RNA, some to proteins, as the substances in which memories are coded. As you remember, these macromolecules can be built in an almost infinite number of ways.

RNA and memory

It is a demonstrated fact that the RNA content of neurons increases with their activity so long as this activity is within normal ranges. Furthermore, it has been found that the RNA content of neurons in the human spinal cord increases from age 3 to age 40. After age 40 the RNA content of these cells remains quite constant until age 55 or 60, when it declines rapidly.

That memory might be specifically encoded in RNA has been proposed as the explanation of certain learning experiments which have been carried out on planarians. As we mentioned, these creatures can learn to solve a *T*-maze. They can also learn a conditioned response. The head of the worm dominates the tail in these responses. If the worm is cut into two pieces, both the head and the tail regenerate the missing parts. Both regenerated worms retain some memory of their prior training. However, if the pieces are allowed to regenerate in pond water to which some of the RNA-digesting enzyme, ribonuclease, has been added, only the dominant, trained head retains its memory. The head that is regenerated by the severed tail performs no better than a totally untrained worm. Another experimenter has claimed that when trained (conditioned) planarians are ground up and fed to untrained ones, the latter can be similarly trained in a much shorter time than normally fed individuals.

Such remarkable reports as these have produced a flurry of related experimentation in many different laboratories. These experiments have been done not only with planarians but also with "higher" animals such as rats and mice. Some workers claim that learning can be transferred from one animal to another with RNA extracted from the trained animals and injected into untrained ones. Other investigators claim to have accomplished the same feat with proteins and even, in one case, a polypeptide

containing 15 amino acids. Some workers have found drugs which, they claim, speed up RNA synthesis and also learning. Unfortunately, in every one of these cases, workers in other laboratories have often been unable to duplicate these results. Perhaps this should not really be too surprising when you consider the many subtle factors that could influence the behavior of an animal like a rat but be overlooked by the experimenter. Furthermore, the transfer of learning that is claimed really involves simply *faster learning* on the part of the recipients than in the controls. Perhaps these extracts and chemicals that are thought to transfer or speed up learning may simply be speeding up the *general activity* of the recipients.

Phases in the acquisition of memory

It is still too soon to say just what the nature of memory is. Perhaps both dynamic processes as well as physical-chemical alterations are involved. This would certainly be consistent with the discovery that the acquisition of a memory seems to occur in at least two distinct steps. In man, for example, damage to the temporal lobes may result in the loss of ability to remember new learning for more than about one hour. Such damage has no effect on memories acquired in the years before the damage occurred. Mental patients undergoing electroshock therapy are unable to remember events that occurred just prior to the treatment, but the memory of earlier events is unimpaired. In goldfish and mice, the application of chemicals that inhibit protein synthesis has been shown to prevent the *acquisition* of long-term memories but not of short-term ones. However, inhibition of protein synthesis does not inhibit the *recall* of old memories. This has been demonstrated by training a group of mice to choose one (e.g. the left) arm of a *T*-maze. Three weeks later they are retrained to choose the other (the right). Then some are given an inhibitor of protein synthesis and others simply saline. When tested three days later, the mice whose protein synthesis had been suppressed chose the left arm; the mice that received saline went right!

It should be apparent from this chapter that we know a great deal more about the simple forms of behavior, such as the simple reflex and the conditioned response, than we do about the more elaborate processes involved in insight and reasoning. We can be reasonably certain that further study will gradually tell us more about the mechanisms of these more elaborate mental activities so characteristic of man. While we look forward to these discoveries, we must also be warned by the memory of the ways in which past scientific discoveries, such as nuclear fission, have sometimes been used. The knowledge of how men's minds function is knowledge that will have to be used with great wisdom.

EXERCISES AND PROBLEMS

1. Design an experiment to show *whether or not* scout bees can communicate to other foragers information about the direction of a food source 200 yards from the hive.
2. How could you prove that the odor receptors of the honeybee are located on the antennae? Describe your procedure in detail.

3. If a man is shocked through his foot, simple reflex withdrawal of the foot will occur. Describe how this reflex might be "conditioned." What changes in the functioning of the nervous system occur during this process?
4. On March 20th, the sun rises in the east at 6:00 a.m. and sets in the west approximately 12 hours later. At 9:00 a.m., a scout bee does the tail-wagging dance so that the straight portion of the dance is 135° to the right of the vertical. In which direction is the food that it is reporting?
5. How will a scout bee dance in the hive at noon on March 20th when it has discovered food 1000 m to the north of the hive?
6. How will it dance when food has been discovered 5000 m northeast of the hive?
7. Summarize the various methods of intraspecific communication found among animals.
8. Distinguish between a tropism and a taxis.
9. Design an experiment to show whether a dog can discriminate between two adjacent whole notes on the piano.
10. Design an experiment to show whether ants have any color vision.

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30

THE IMMUNE RESPONSE

Introduction

What are Antibodies?

What do Antibodies do?

Antigens

How are Antibodies Elicited?

The Secondary Response

The Basis of Antibody Diversity

Transplantation and the Uniqueness of the Individual

Cancer and Immune Surveillance

Auto-Immunity

Coda

30.1 INTRODUCTION

Immunity is a form of learning

For many centuries people have been aware that certain diseases never strike the same person twice. A pockmarked survivor of one smallpox epidemic could safely handle patients during a subsequent epidemic. As a result of contracting the disease, some change had occurred within his body making him henceforth *immune* to the disease. The responsiveness of the individual had been permanently altered as a consequence of his experience—a form of learning.

Specificity of immunity

What is the nature of this change? In Chapter 19, you learned that pneumococci, the agents that cause bacterial pneumonia, exist in a large number of different types (I, II, III, etc.). A survivor of a bout of Type III pneumonia becomes immune to pneumonia of *that type only*. If one injects no more than two or three Type III pneumococci into the bloodstream (not their usual habitat) of a mouse, the bacteria proliferate quickly and the mouse soon dies of septicemia (blood poisoning). However, if a small amount of blood serum from an immune human is injected along with hundreds of thousands of Type III pneumococci, the mouse remains healthy. The blood of a human who has never been sick with *Type III* pneumonia has no such protective effect. This experiment tells us that at least one manifestation of immunity is to be found in the blood. We say that the immune serum contains **antibodies** against the disease.

30.2 WHAT ARE ANTIBODIES?

Antibodies are immunoglobulins

Prominent among the various components of the blood are proteins such as albumin, fibrinogen, and globulins (see Section 13.13). There are a number of techniques by which these proteins can be isolated from one another (see Section 3.13). When this is done to immune serum, the fraction containing the globulins is the only fraction that can, to pursue our example, protect a mouse from death by Type III pneumococci. These antibodies (and all others) are, then, serum globulins.

Thanks to the pioneering efforts of Sanger (see Section 4.3), it is now possible to determine the primary structure of a protein, that is, its precise sequence of amino acids. This has been done for insulin (Fig. 4.13), hemoglobin (see Section 19.4), ribonuclease, the coat protein of the virus MS2 (Fig. 19.19), and many others. Why not do the same for an antibody?

The heterogeneity of antibodies

Unfortunately, it turns out that there is not just a single kind of globulin molecule that is an antibody against, for example, Type III pneumococci. There are, in fact, many of them. Insulin, hemoglobin, ribonuclease, and the MS2 coat protein all consist of just a single kind of molecule which can be isolated and analyzed. Not so for antibodies. Isolation of the antibodies against Type III pneumococci presents one with a mixture containing a number of *different* protein molecules, each with its own primary structure. Until quite recently, no techniques existed for further fractionation of this mixture so that one or more purified single components could be analyzed.

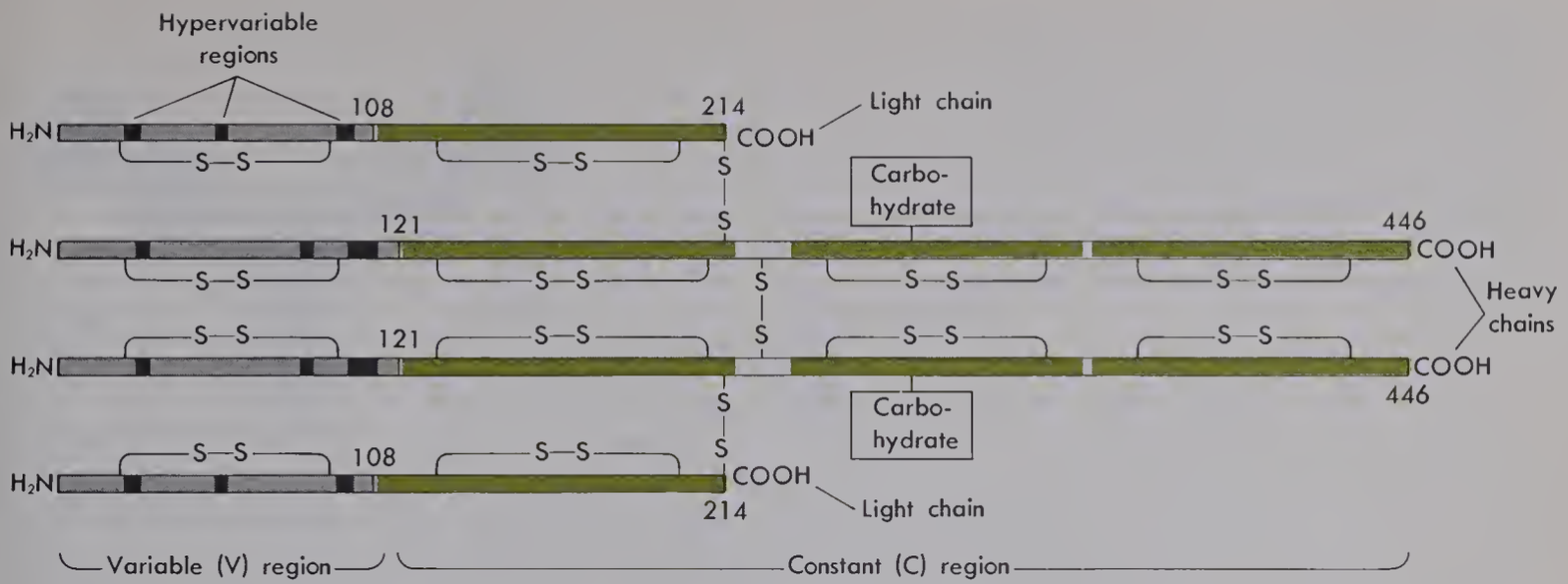


Fig. 30.1 Polypeptide chain structure of an antibody molecule. The numbers indicate the number of amino acids (counting from the amino terminal, $—NH_2$, end). The two light chains are identical as are the two heavy chains. In the intact molecule, the chains are folded so that each cysteine is brought close to the partner with which it forms a disulfide (S-S) bond (cf. Fig. 4.13). In a given species, different antibody molecules (of the type shown here) have the same or almost the same sequence of amino acids in the constant regions, but show marked differences in the variable regions. These differences are especially pronounced in the hypervariable regions.

Multiple myeloma

The solution to this dilemma came from the realization that a rare form of human (and mouse) cancer involves the antibody-producing system. A victim of this disease, called multiple myeloma, produces large amounts of a *single* type of antibody molecule. This is not (so far as we know) the result of an infection but, rather, another example (see Section 23.2) of a cell entering upon a period of unchecked proliferation. In this case, the cell is an antibody-secreting cell, and the **clone** (see Section 18.3) that develops yields a single protein product.

Even though one doesn't know what the antibody is an antibody *against,* the large amounts and purity of this material have enabled a number of research workers to slowly piece together a picture that we have reason to believe represents the basic structure of many kinds of antibody molecules.

Structure of the antibody molecule

Figure 30.1 shows the general structure of myeloma proteins as worked out by Edelman and his colleagues. The molecule consists of (1) two long polypeptide chains (the heavy chains) containing approximately 446 amino acids and identical to each other; (2) two short (approximately 214 amino acids) chains (the light chains), which are also identical to each other; and (3) some carbohydrate attached to the heavy chains. The heavy chains are attached to each other and to the light chains by S—S bridges. There are also S—S bridges *within* both the light and heavy chains and these force the molecule into a number of compact, globular regions (Fig. 30.2).

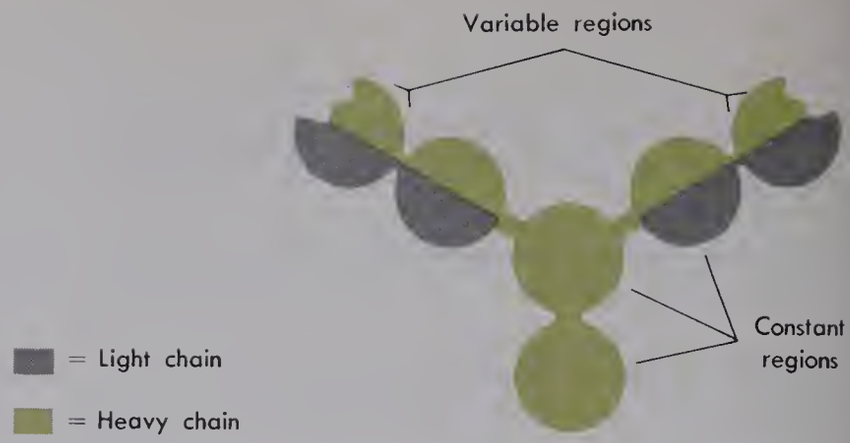


Fig. 30.2 Schematic representation of the three-dimensional structure of an antibody molecule. The specificity of an antibody, i.e. its ability to bind a particular antigenic determinant, resides in the globular domains created by the variable regions of the light and heavy chains. The domains formed by the constant regions accomplish other functions of the molecule.

The constant (C) regions

The analysis of a number of *different* human myeloma proteins has revealed some curious uniformities. Half of each light chain and three-quarters of each heavy chain (the —COOH terminal portion in each case —Fig. 30.1) have virtually identical sequences in every human myeloma protein. These regions are thus called the constant (C) regions. The constant region of the heavy chains consists of three very similar sequences (each containing about 108 amino acids) arranged in tandem. These sequences are also similar to that found in the constant region of the light chain.

The variable (V) regions

The situation is quite different in the amino (NH_2—) terminal (Fig. 30.1) segment of each light and heavy chain. Here each human myeloma protein examined shows different amino acids in many of the positions. Because of the variety of amino acid substitutions found, these portions of the chains are called the variable (V) regions. The sequence variability in the V region is especially pronounced in three areas. These so-called *hypervariable* regions are located approximately at positions 24–34, 50–56, and 89–97 in light chains and 31–37, 86–91, and 101–110 in heavy chains (Fig. 30.3). In the next section, we shall see evidence that these hypervariable regions are crucial to antibody function.

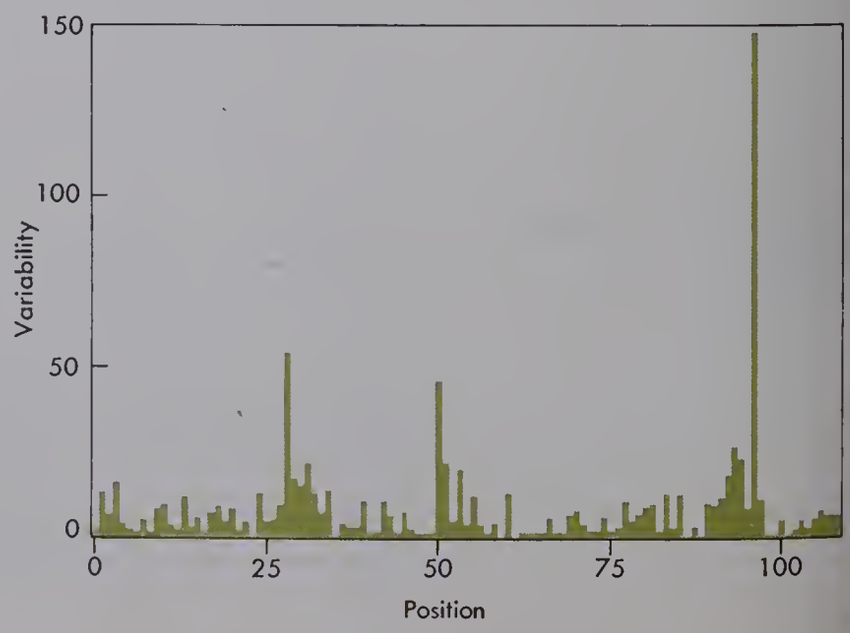


Fig. 30.3 Degree of variability found at each position in the variable (V) region of a large number of light chains derived from myeloma proteins. Where only one amino acid has ever been found at a given position, the variability is 1. If each of the 20 amino acids occurred with equal frequency at a given position, the variability would be 400. The three regions with high variability are the hypervariable regions.

You might wonder if myeloma proteins—the result of a disease process—are truly representative of the structure of antibodies. Recently, rabbit antibodies of *known* specificity (e.g. antibodies against Type III pneumococci) have been prepared that are as homogeneous as myeloma proteins. The primary structure of these antibodies fully confirms the validity of the model of antibody structure based on the myeloma proteins.

30.3 WHAT DO ANTIBODIES DO?

Pneumococci are coated with a polysaccharide

The type (III, IV, VIII, etc.) of a pneumococcus is determined by the molecular structure of the polysaccharide capsule that surrounds the organism. The capsule can be removed from the bacterium and the polysaccharide purified. When a solution of pure Type III polysaccharide is added to a solution of anti-Type III pneumococcal antibodies, a dense white precipitate forms (Fig. 30.4). If the quantities used are carefully chosen, the solution remaining above the precipitate can then be shown to be completely devoid of either antibody or polysaccharide. All the polysaccharide and all the antibodies that were present initially are found complexed together in the precipitate. Antibodies, therefore, combine with the mate-

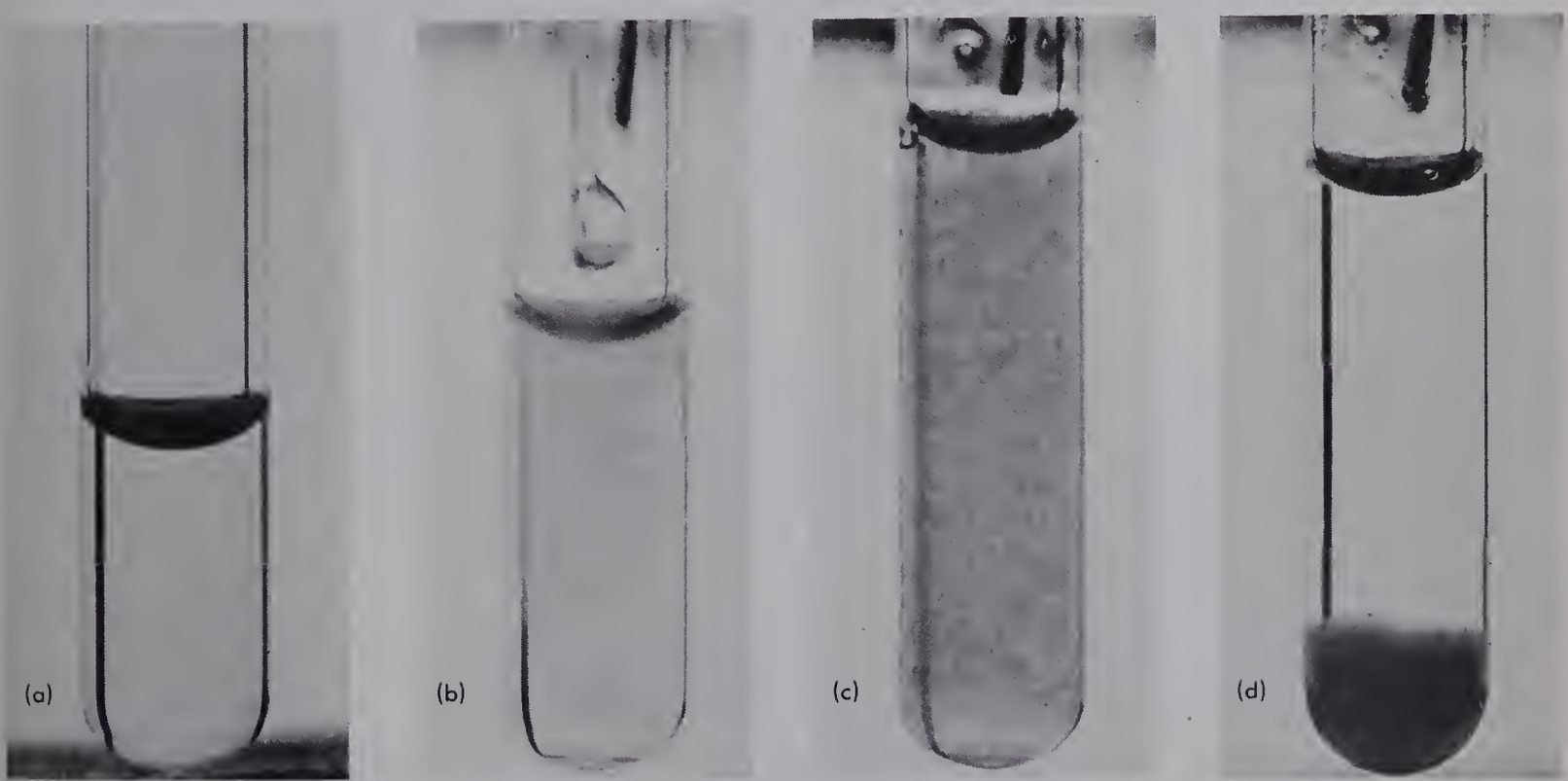


Fig. 30.4 Antigen-antibody interaction. The test tube at the left (a) contains a solution of antibodies to the Type III pneumococcal polysaccharide. A solution of the polysaccharide is added (b) and the formation of insoluble antigen-antibody complexes is revealed by the almost instantaneous appearance of turbidity (c). After an hour, the complexes settle out as a precipitate (d). If the proportion of antigen to antibody in the mixture was selected properly, the fluid above the precipitate will now be devoid of either.

rial that elicited them. We call this material the **antigen.** This behavior of antibodies is like that of an enzyme when it combines with its substrate (Fig. 6.16).

The Type III pneumococcal polysaccharide is an enormous polymer consisting of a linear array of thousands of glucose and glucuronic acid (a close relative of glucose) units alternating with each other (Fig. 30.5). If small lengths of this molecule (e.g. a disaccharide, trisaccharide, etc.) are added to anti-Type III antibodies, combination again occurs. However, the combining ability of the antibodies is not exhausted. They are still able to combine with the intact Type III polysaccharide. However, if we try lengths containing five to seven sugar units, the antibody is no longer capable of combining with the natural polysaccharide. This suggests that the antibody molecules combine with a portion of the polysaccharide that is only five to seven sugar units long (or approximately 24–30 A). It is interesting to note that antibodies directed against other types of molecules (e.g. proteins) also unite with only a portion of the molecule, and that portion is of similar length. Thus it is not enough to say that antibodies combine with antigens, but rather they combine with a small part of the antigen, the so-called **antigenic determinant.**

The antigenic determinant

We assume that the antigenic determinant fits into a groove or cleft on the antibody molecule. This cleft has a shape complementary to that of the determinant (Fig. 30.6). Thus we are dealing with the same sort of lock-and-key arrangement that we postulated for enzyme-substrate complexes (Fig. 6.16). It should be noted that the hypervariable regions of the light and heavy chains are located so that all three of them could participate in the making of a cleft, i.e. the antibody-combining site (Fig. 30.7).

The antibody-combining site

Most antigens are far more complex in structure than the Type III polysaccharide. The toxin produced by the diphtheria bacillus and the outer coat of a polio virus are just two examples of protein antigens that have several antigenic determinants. Exposure to these materials will elicit the formation of antibodies against each determinant. While this accounts for only a part of the story of antibody diversity, it is no wonder that early workers were frustrated in their search for a single antibody molecule to analyze.

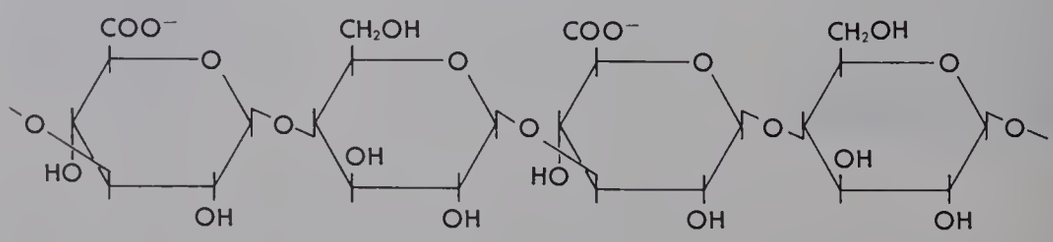


Fig. 30.5 Molecular structure of the polysaccharide capsule surrounding Type III pneumococci. The molecule is an extremely long, unbranched polymer consisting of alternating units of glucose and its relative glucuronic acid (with the —COO^- group).

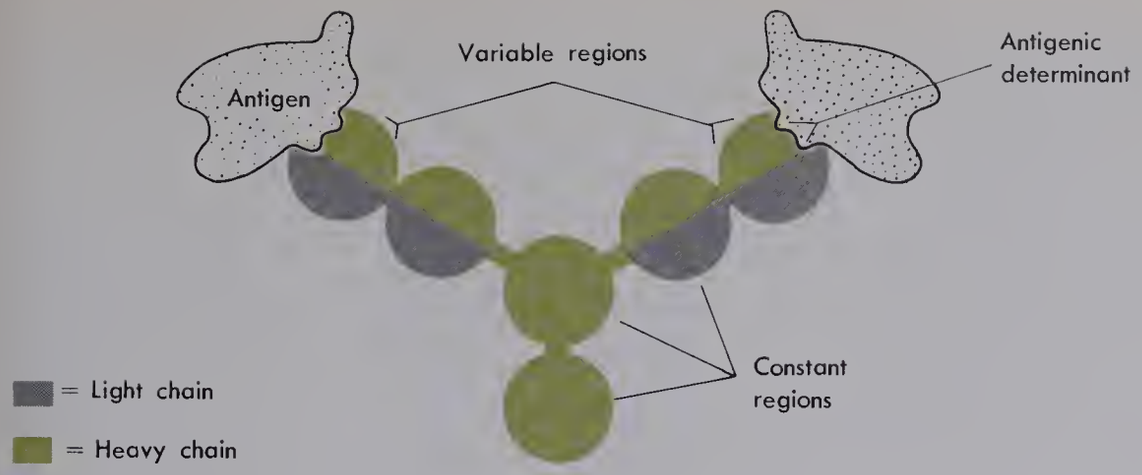


Fig. 30.6 Schematic representation of the binding of two antigen molecules to the antigen-combining sites in the variable domains of the antibody. The V regions of both the light and heavy chains participate in forming the antigen-binding site. The two sites in a molecule are identical.

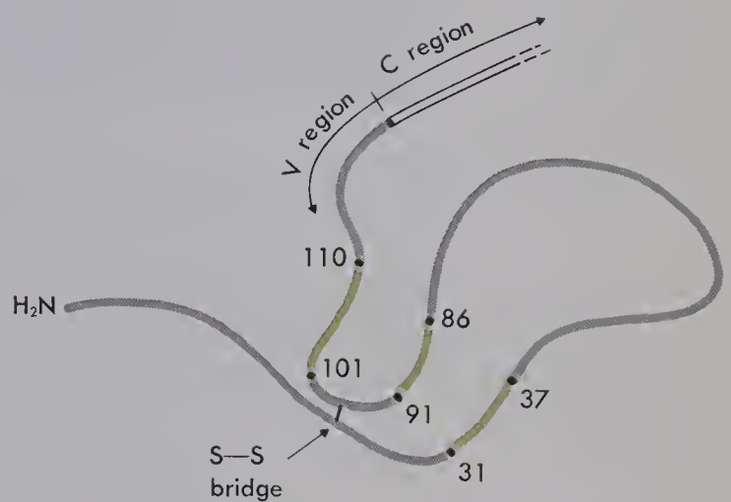


Fig. 30.7 All three hypervariable regions (color) of the heavy chain may lie close to one another and, if so, could participate in forming the antigen-combining site of the antibody. The same holds true for the hypervariable regions of the light chains, which also contribute to the formation of the combining site.

Antibodies may enhance phagocytosis

Of what biological significance is the union of antigen and antibody? Once the union occurs, the body has several mechanisms by which it can deal with the antigen-antibody complex. One of these is shown in Fig. 30.8. The capsule that surrounds pneumococci greatly interferes with their being engulfed by phagocytes. In fact, it is this property of the capsule that makes pneumococci so virulent. You remember (see Section 19.1) that capsule-less pneumococci (R forms) do not cause disease. If the appropriate anti-pneumococcal antibodies are present, they combine with the capsule and render the cells easy to engulf by endocytosis. In the days before the discovery of antibiotics, the start of antibody production by the immune system of the patient marked the turning point in the progress of the disease.

Life cannot be maintained for long in the total absence of immune responsiveness

Another way of assessing the importance of antibodies is to examine the fate of those rare babies born without any immune system. For the first six weeks of life, they get along fine thanks to their mothers' antibodies, which they had received across the placenta. Soon this protection becomes inadequate, however, and the babies fall victim to raging bacterial and viral infections. The former can be aided by antibiotics but not the latter and,

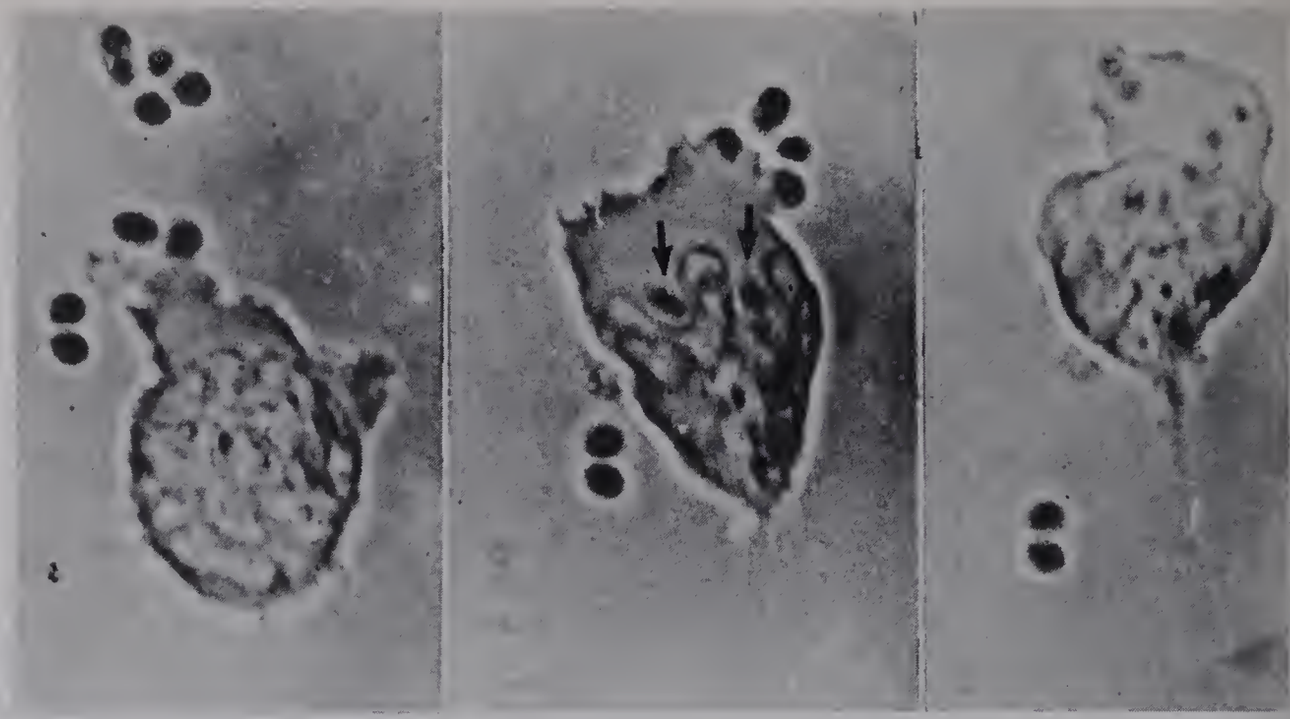


Fig. 30.8 Phagocytosis. Left: a neutrophil extends a pseudopod towards two pneumococci. Center: these bacteria have now been engulfed (arrows) and the neutrophil is beginning to engulf four more pneumococci at the upper right. Two pneumococci were spared (right). Antibodies to the polysaccharide capsule surrounding the pneumococci were added to enhance phagocytosis. (From W. B. Wood, M. R. Smith, and B. Watson, *Journal of Experimental Medicine,* **84,** 387, 1946.)

until quite recently, death was inevitable. Now, however, techniques have been developed so that some of these victims can be given the machinery for developing immunity and their lives saved.

30.4 ANTIGENS

Noninfectious antigens

Although we have up to now emphasized immunity against infectious organisms, the immune system is by no means limited to dealing with such material. The immune system is capable of making antibodies against a variety of noninfectious materials such as ragweed pollen, the active component of the poison ivy plant, and the Rh antigen, to name three troublesome examples. The Rh antigen is located on the surface of the red blood cells of "Rh positive" individuals. Before the discovery of the Rh antigen, "Rh negative" women (whose RBC's lack the antigen) were sometimes inadvertently given transfusions of Rh positive blood. Unknown at the time, they responded by making anti-Rh antibodies. If such a woman later became pregnant with an Rh positive baby (the trait is inherited as a dominant), the fetus would often become dangerously anemic as the mother's antibodies (which cross the placenta) attacked its red cells.

Antigens are macromolecules

Are there, then, limits to what can serve as an antigen? We have seen that polysaccharides and proteins serve as excellent antigens. Somewhat less effective but still antigenic are nucleic acids, the third main category

of macromolecules. On the other hand, small molecules (e.g. glucose) do not elicit antibody formation. Size, therefore, seems to be one requirement for provoking an immune response. But what about the small molecule that causes poison ivy poisoning? What probably happens in this case is that the molecule first joins with some protein in the skin, and it is the resulting complex that becomes antigenic.

Although only macromolecules stimulate the immune mechanism, the antibodies that are formed combine with just a small portion, the determinant, of the antigen. The interaction between the two is remarkably precise. Our immune system can make antibodies that discriminate between pig insulin and human insulin (where only one amino acid out of 51 is different) and even between galactose and glucose present in a determinant (isomers that are identical except for the orientation of an —H and —OH group—see Fig. 4.2).

"Self" vs. "nonself"

There is one group of macromolecules which do not act as antigens. These are macromolecules that are normal components of the body. We make antibodies in response to injections of albumin from cows but not of albumin from humans. The reason is that the albumin molecules in one human have the same structure as those in another. Thus we must add the additional criterion that a macromolecule can act as an antigen only when it is "foreign" to the body.

Although humans share many identical macromolecules, each of us has others that are *unique* to us (unless we have an identical twin). This is why grafts of skin (or organs) from one individual to another are attacked by the recipient's immune system while grafts of skin from one part of the body to another are not. The immune system thus discriminates between "self" and "non-self."

Immunological tolerance

It can be fooled. Fetal calf twins share a common placenta and thus, until birth, a common blood system. Although developed from separate fertilized eggs and thus not in any sense genetically identical, such animals act like identical twins in their inability to make antibodies against each other's macromolecules. Transplants of skin from one to the other "take" just as well as they do between identical twins (see Section 15.8).

Artificial induction of tolerance

Nature's experiment with cattle twins can be duplicated with other animals. Injections of foreign molecules into a newborn mammal—before its immune machinery has been set up—will create a situation of **tolerance.** Later, when the animal becomes able to make antibodies, it will fail to do so against the material given to it at birth. It has accepted such material as "self."

30.5 HOW ARE ANTIBODIES ELICITED?

Both phagocytes and lymphocytes are usually needed to elicit antibodies

An enormous amount of research has gone into trying to piece together the steps that take place between the introduction of an antigen into the body and the start of antibody synthesis. It is now possible to carry out the entire process *in vitro* (in the "test tube"). Two kinds of cells are usually required, phagocytes that are the tissue equivalent of monocytes (Fig. 13.9), and

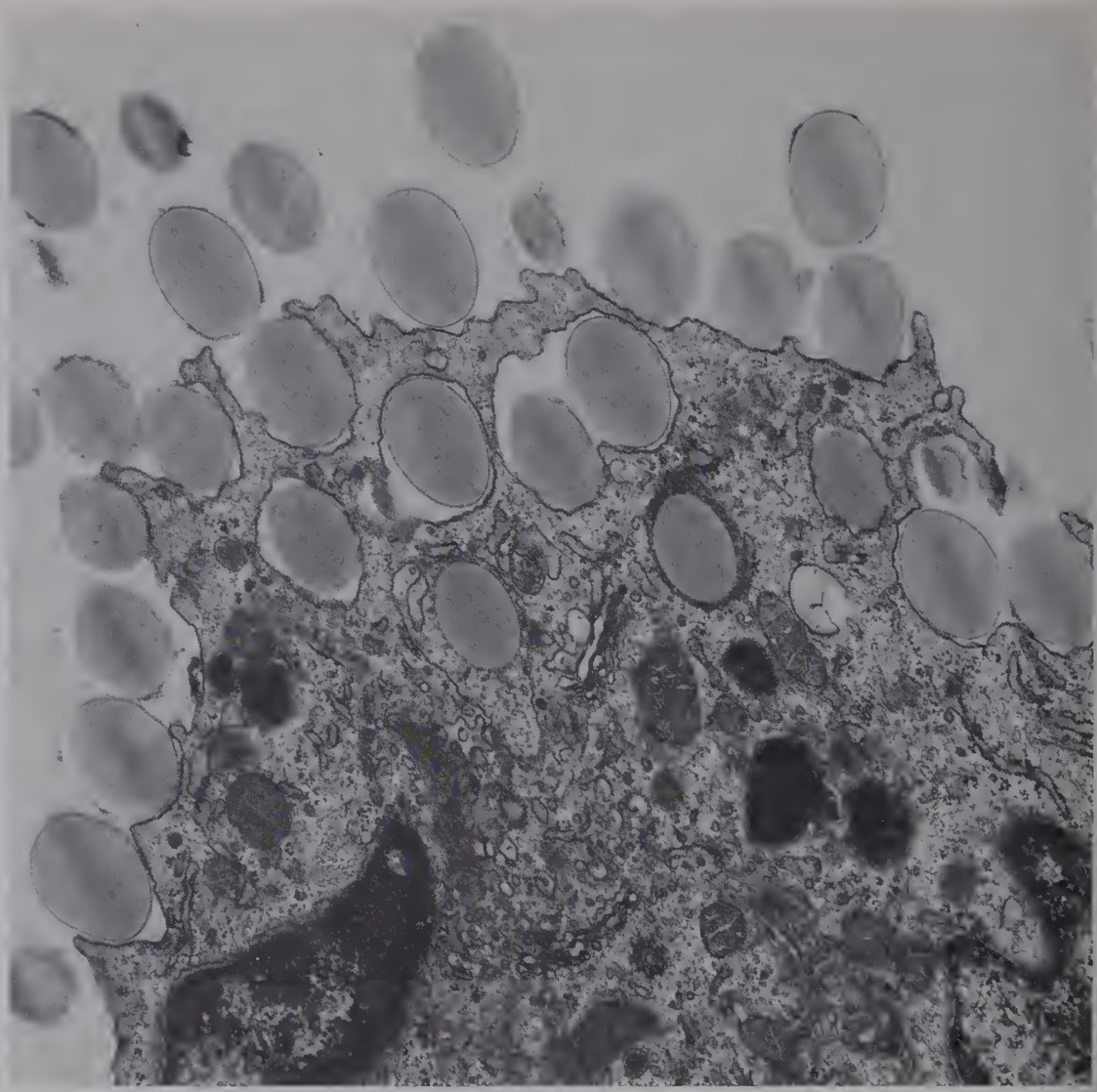


Fig. 30.9 Guinea pig phagocyte ingesting polystyrene beads. Several beads are already enclosed in vacuoles, while others are in the process of being engulfed. (Can you find a centriole?) (22,000 X, courtesy of Dr. Robert J. North.)

lymphocytes. The first step in the process is the engulfment of the antigen by phagocytes (Figs. 30.8 and 30.9). What happens next is still uncertain, but in some way the phagocytes interact with lymphocytes.

You might assume, as many did at one time, that the phagocytes instruct lymphocytes how to go about the next step in antibody synthesis. But such is surely not the case. There is a good deal of evidence that *before* the introduction of an antigen, the body already has lymphocytes capable of recognizing that antigen. The lymphocytes demonstrate this capability by actually combining with the antigen, possibly after it has been processed in some way by phagocytes. The reaction between lymphocytes and antigen occurs at receptors on the cell surface that are probably identical (at least in the combining site) to the antibody molecules that will ultimately appear in the blood.

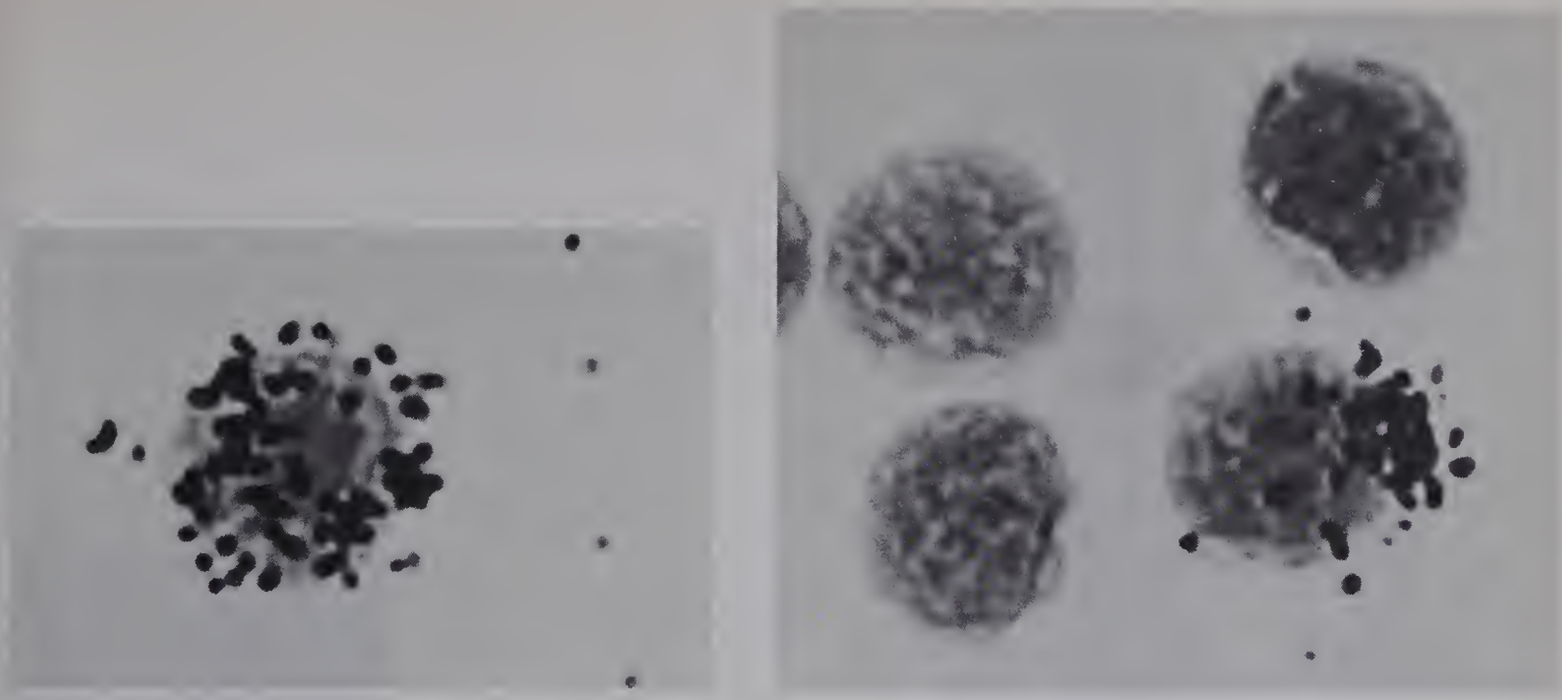


Fig. 30.10 Left: autoradiograph of a lymph node cell to which molecules of a radioactive antigen have adhered. This cell was taken from an *unimmunized* mouse. Right: after 15 minutes of incubation at 37°C, the radioactivity is clustered in one region. This indicates that the cell's antigen-binding receptors are free to migrate over the surface of the cell. Note the other cells that have not bound any of the radioactive antigen. Presumably they have receptors for other antigens. The work pictured here represents an extension of the findings of Ada and Byrt. (Courtesy of E. Diener from E. Diener and V. H. Paetkau, *Proceedings of the National Academy of Sciences, U.S.A.*, **69**, 2364-2368, September, 1972.)

The experiments of Ada and Byrt

Let us examine two of the experiments that lead to this remarkable conclusion. A number of years ago, the Australian immunologists Ada and Byrt prepared a highly radioactive antigen. When this material was mixed with lymphocytes taken from mice that had never been exposed to the antigen, it stuck to a small percentage (0.02%) of the cells (Fig. 30.10). The radioactivity was so great that these cells (but *only* these) were killed. When the remaining cells of the original population were returned to a mouse whose own immune system had been destroyed (by x-rays), that mouse was *incapable* of synthesizing antibodies to the original antigen. However, it was perfectly capable of synthesizing antibodies against any other, even closely similar, antigens. Thus it appears that the unimmunized mouse already has a few lymphocytes carrying antibody-like receptors that are ready to detect and interact with the corresponding antigenic determinant when and if it appears.

Edelman's demonstration of antibody-like receptors on the surface of lymphocytes

In 1972, Edelman and his colleagues at the Rockefeller University demonstrated the existence of antigen-reactive cells in nonimmune mice by another technique. They attached an antigen to the surface of nylon fibers and then placed the fibers in a dish containing spleen cells (mostly

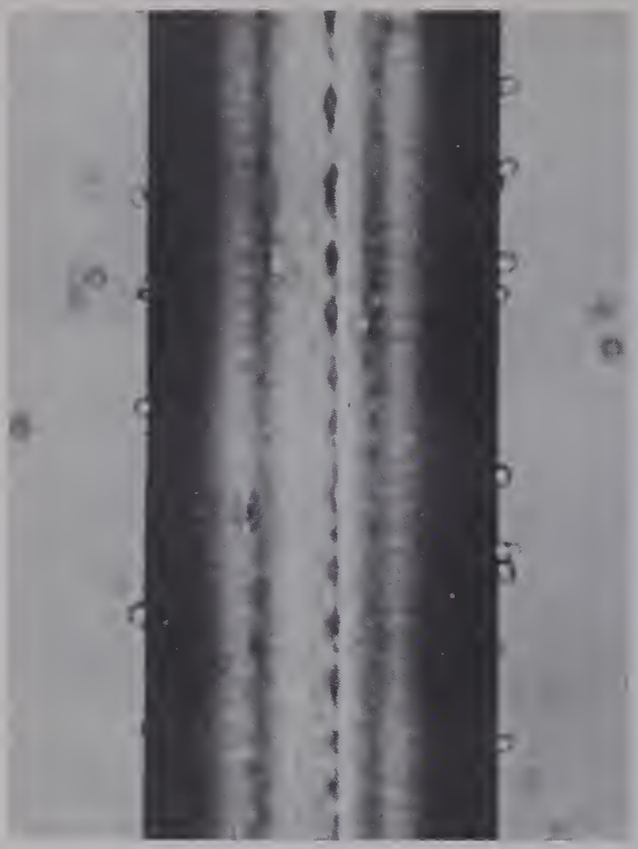


Fig. 30.11 Mouse spleen cells (lymphocytes) adhering to the surface of an antigen-coated nylon fiber. No cells would stick (1) if the nylon fiber had no antigen attached to it; (2) if the cells were first exposed to the same antigen in solution (thus "filling" all the receptors); or (3) if the cells were first exposed to antibodies against mouse antibodies (thus suggesting that the receptors are, indeed, antibody molecules). (Courtesy of G. M. Edelman from U. Rutishauser, C. F. Millette, and G. M. Edelman, *Proceedings of the National Academy of Sciences, U.S.A.*, **69**, 1596, 1972.)



Fig. 30.12 In this photo, a mouse spleen cell stuck to an antigen-coated nylon fiber has been exposed to sheep red blood cells coated with the same antigen. The results (like those in Fig. 30.10) suggest that the antigen-binding receptors of the cell are distributed over its entire surface. Untreated sheep red blood cells would not stick to this mouse cell. (Courtesy of G. M. Edelman, from the same article as that of Fig. 30.11.)

lymphocytes) from an unimmunized mouse. A small percentage (about 1%) of these stuck to the fibers, indicating the presence of complementary antibody-like molecules on their surface (Fig. 30.11). In fact, once stuck, the rest of the cell surface was still capable of interacting with additional antigen (Fig. 30.12). However, if the cells were first treated with antigen *or* with antibodies directed against mouse antibodies ("anti-antibodies") no sticking occurred.

The clonal selection theory

Once an antigen has attached itself to the receptor of the appropriate lymphocyte, that cell becomes stimulated to divide (Fig. 30.13). With repeated mitotic divisions, a *clone* of cells capable of synthesizing that particular antibody develops. The idea that antibody-producing clones arise not by instruction but as a result of the selective stimulation of *preexisting* cells is called the clonal selection theory. The metabolic demands of mitosis are so great that not much antibody is synthesized during this period. But a time comes when more and more of the cells in the clone stop dividing and begin tooling up for massive antibody production. A

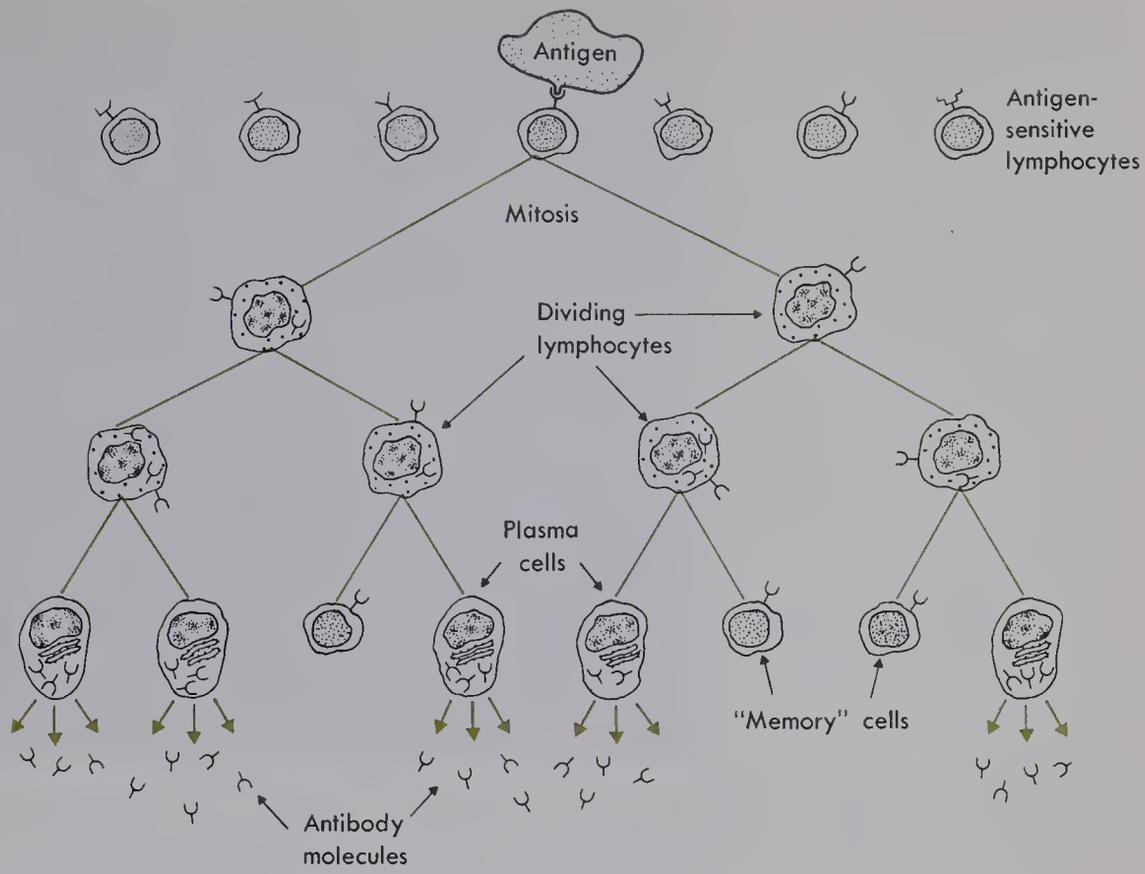


Fig. 30.13 Schematic representation of the clonal selection theory. Clonal selection leads to the production of antibody-secreting plasma cells *and* an enlarged pool of specific antigen-sensitive ("memory") cells. The exact form in which antigen is presented to the antigen-sensitive cells is not known.

large "rough" endoplasmic reticulum develops (see Section 5.7) and soon the cell is synthesizing one major protein component: the antibody. Such a cell is called a **plasma cell.** After assembly in the Golgi apparatus (where some of the carbohydrate is added), the antibody is secreted from the cell (by exocytosis).

Plasma cells secrete antibody

Figure 30.14 shows plasma cells taken from the spleen of a rabbit immunized with Type III pneumococci. The cells were treated *first* with the Type III polysaccharide, *then* with anti-Type III antibodies that had been coupled to a fluorescent dye. Viewed under ultraviolet light, each cell synthesizing anti-Type III antibody glows brilliantly.

The role of the lymph nodes

Antibody production occurs in the spleen, in the bone marrow, and, perhaps most important, in the lymph nodes. Lymph nodes are ideally situated to encounter antigens that enter the body through the skin, lungs, and intestine. Processing, as they do, the tissue fluids from these (and most other) organs, they are quickly exposed to foreign material. Not only do they prevent such material from reaching the bloodstream, but they have



Fig. 30.14 Antibody-containing plasma cells from the spleen of a rabbit immunized with Type III pneumococci. These cells were first treated with the antigen, Type III pneumococcal polysaccharide, and then with anti-Type III antibodies attached to a fluorescent dye. Viewed under ultraviolet light, those plasma cells manufacturing anti-Type III antibodies glow brightly. (Courtesy of Dr. Albert H. Coons.)

all the machinery for mounting an immune response. The secreted antibodies are transported in the lymph that drains from the node and enter the blood at the subclavian veins (look back at Fig. 13.14).

30.6 THE SECONDARY RESPONSE

After recovery from an infection, the concentration of antibodies against the infectious agent gradually declines over a period of weeks, or months, or even years. A time *may* come when no further antibody can be detected. Does this mean that the individual is once again in danger of contracting the disease? In many cases, no. Whereas the production of a significant level of antibodies upon first exposure to the antigen takes, on average, four or five days, reexposure—even after many years—calls forth a much faster and longer response to the antigen. This is called the **secondary response** (Fig. 30.15).

Memory cells

What mechanism is at work here? The key seems to be the presence, during the intervening period, of a larger population of antigen-sensitive lymphocytes ("memory" cells) than existed before the primary response. Probably during the initial development of clones, some of the progeny cells neither went on dividing nor developed into plasma cells. Instead,

they reverted to small lymphocytes bearing the same antibody molecules on their surface that their ancestor had (Fig. 30.13). In this way, the total number of such antigen-sensitive cells is substantially increased and the foundation laid for a bigger (and thus sooner detectable) response the next time the antigen appears. This idea is supported by the discovery that when spleen cells from *immunized* mice are mixed with the appropriate antigen-coated nylon fibers, the number of cells that stick *is* increased (and these are *not* antibody-*secreting* cells).

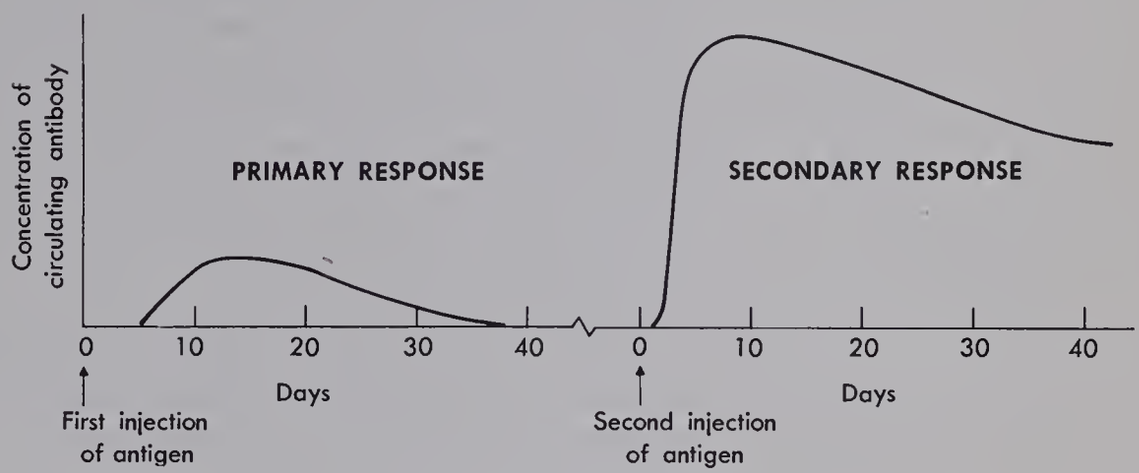


Fig. 30.15 Antibody production during the course of a typical primary (left) and secondary (right) response. The actual time course varies with such factors as the nature of the antigen and the way in which it is administered to the animal.

The life span of lymphocytes

Not only does the secondary response require a pool of memory cells, but these must have long life spans if we assume that they will never divide again unless they encounter the appropriate antigen. A number of studies have shown that among the lymphocytes that circulate from lymph nodes to the blood and back again are some that are, indeed, long-lived. There is evidence in humans that some small lymphocytes live as long as 20 years.

Specificity of the secondary response

Immunological memory is exceedingly precise. As you are probably painfully aware, influenza is caused by a variety of closely related viruses. From time to time, new virus variants (e.g. the "Hong Kong flu") appear and sweep through the population. In 1918, a worldwide flu epidemic killed an estimated 20 million persons. When survivors of that epidemic are exposed today to influenza virus, the antibodies they produce are not as effective against the present-day virus as they are against the virus with which they were first infected in 1918. Although no one even knew in 1918 that flu was caused by a virus, we can now identify the 1918 virus simply by examining the specificity of the antibodies that survivors produce today in their secondary response.

The secondary response thus represents a long-term alteration in the responsiveness of the organism as a result of its experience. Is, then, immunological memory so different from memory stored in the central nervous system?

Vaccines

Immunological memory provides the main foundation for the use of **vaccines.** These are preparations of infectious or toxic agents that have

been altered so as not to cause disease. The alteration is not, however, so drastic that antibodies produced in response to the altered material will not protect against the unaltered, disease-producing agent. This usually means that the alteration must leave some of the antigenic determinants intact. Formaldehyde is often used to render bacteria and viruses noninfectious without damaging all the antigenic determinants. The Salk polio vaccine is prepared in this manner.

The first scientific vaccination was performed by the English physician Edward Jenner in 1796. He made medical history by inoculating a human subject with material squeezed from the sores of a girl afflicted with cowpox and then, a few months later, with material from the sores of people afflicted with the dread disease smallpox. His subject remained perfectly well. Jenner performed this daring experiment because he (and others) had long noted that milkmaids who had contracted cowpox from their charges were protected from the ravages of smallpox. Today, we realize that they gained this protection by developing antibodies which were effective not only against the cowpox virus but against the closely related smallpox virus as well.

You have probably been given smallpox vaccine, polio vaccine, and vaccines against tetanus, diphtheria, and whooping cough as well. In the case of tetanus and diphtheria, it is the toxins we must guard against. Their vaccines thus consist of chemically altered toxins, called **toxoids.** Vaccines against typhoid fever, typhus fever, bubonic plague, Asiatic cholera, and yellow fever are all available for people who live or travel in regions where these diseases are present.

30.7 THE BASIS OF ANTIBODY DIVERSITY

How many kinds of antibody molecules can we make?

Our discussion of the immune response certainly makes the system seem an open-ended one. There seems to be no limit to the number of different antigenic determinants against which the system can operate. But is there a limit to the number of different kinds of antibody molecules that can be synthesized? Probably so, but just what it is, at present is anyone's guess. Not only does a given antigen give rise to many different antibody molecules (as we have seen), but even a single antigenic determinant will elicit the production of a diverse population of antibody molecules. But these different antibody molecules are not all equally effective in combining with the determinant. They represent a spectrum extending from those that do so exceedingly well to those that do so poorly. Presumably this reflects the degree of *precision of fit* between the shape of the antigenic determinant and the shape of the combining site of the antibody. But could not some of the antibody molecules with a poor fit to that determinant have a better fit to some other determinant? It is quite unlikely that all the 1% or so of nonimmune mouse spleen cells that stick to an antigen-coated fiber are sticking equally tightly and, in fact, probably the interaction for most of them is not strong enough to trigger a proliferative response. Most antibodies react to some degree with a variety of related determinants. So,

as a first approximation, the immune response need not be responsible for synthesizing an unlimited number of different molecules. What the limits are, however, is as yet unknown.

Antibodies are proteins, and we have no reason to believe that they are synthesized any differently from any other protein. That is to say, we must assume that the cell nucleus contains DNA coding for that antibody, and that this DNA is transcribed into complementary messenger RNA molecules from which the polypeptide chains are transcribed (see Section 19.5).

Do we inherit a gene for each antibody molecule?

But do we inherit a gene for each antibody that we can make? Let us assume that we can synthesize 10,000 different antibody molecules (maybe the figure is larger, maybe it is smaller). Among this population would be found only about a dozen different kinds of constant regions. Our problem, then, is one of accounting for the diversity of the variable (V) regions, in which, after all, the antibody specificity (the combining site) resides. (But if vertebrates do, indeed, have many genes for V regions and only a dozen genes for C regions, we will also have to look for a mechanism by which their products end up in a single polypeptide; i.e. our old rule of one gene = one polypeptide will have been modified to two genes = one polypeptide!) We might not even need 10,000 different V region genes to make 10,000 different antibodies. Heavy chains and light chains are synthesized separately and the V regions of both participate in making up the combining site. Thus only 100 different kinds of light chains combining at random with 100 different kinds of heavy chains would give us 10,000 possible combinations. There is evidence, however, that not all light chains and heavy chains combine with equal ease, and we should perhaps raise the number to account for the combinations that would not make functional antibodies of any sort. But even if we raised the figure to 1000 V_L (light chain) and 1000 V_H (heavy chain) genes, the DNA needed to code this information would represent only about 0.02% of the DNA found in each gamete.

Apparently there is no molecular configuration that the organic chemists can devise that cannot serve as an antigenic determinant. But why would we have passed on from generation to generation a gene for a V region that *might* someday meet the corresponding antigenic determinant? Our knowledge of cross-reactivity tells us, however, that a given antibody can interact to some degree with a variety of configurations. The antibody made today to a recently synthesized macromolecule may have been made repeatedly in the past to a similar antigenic determinant introduced into the body on, for example, some parasite.

Are new V regions generated by a somatic mechanism?

The possibility that we inherit a gene for every V region we can make should not lead us to ignore other possibilities. If some mechanism existed for generating new V region sequences during the lifetime of the individual, we would be able to meet our antibody needs with a smaller number of inherited genes. A number of hypothetical mechanisms for the somatic generation of V region diversity have been proposed. These range from mechanisms in which a moderate number of V region genes undergo

internal recombination events (analogous to those occurring in synapsis—see Section 16.7) to produce new sequences, to the postulation that a very small number of V genes undergo point mutations (just like those that in germplasm give rise to mutant offspring). (We have no reason to feel that germ cells are the only ones to undergo mutations simply because it is only by examining *offspring* that the presence of such mutations is ordinarily detected.) According to this second mechanism, each mutation in a lymphocyte V gene that produced a codon calling for a new amino acid could lead to the production of a light (or heavy) chain with one amino acid substitution. If present in, or near, the combining site, this change might produce an antibody molecule complementary to a new antigenic configuration. While there is no shortage of theories to explain the origin of antibody diversity, the data which will unequivocally support one mechanism over all the others are simply not yet at hand.

30.8 TRANSPLANTATION AND THE UNIQUENESS OF THE INDIVIDUAL

The first serious medical attempts at transplanting living tissue from one human to another involved the transfusion of blood into victims who had suffered serious blood loss. Sometimes the procedure was successful; sometimes it precipitated a serious, even fatal, crisis.

The ABO blood groups

In 1900, Karl Landsteiner discovered the explanation for these erratic results. He found that antigenic substances may be present on the surface of red blood cells. We call the ones discovered by him the A and B antigens. People having the first are said to have type A blood; people with the second have blood type B. Some people have both antigens (type AB) and others (type O) have neither (Fig. 30.16).

When red blood cells carrying one or both antigens are brought in contact with the corresponding antibodies, they agglutinate, i.e. clump together (Fig. 30.17). Each individual usually has antibodies against those red cell antigens that he lacks. Figure 30.16 shows this and illustrates which

Blood group	Approx. % in U. S. population	Antigens on RBC's	Antibodies in plasma	Can donate to	Can receive from	Genotypes
A	42	A	Anti-B	A, AB	O, A	I^AI^A or I^Ai
B	10	B	Anti-A	B, AB	O, B	I^BI^B or I^Bi
AB	3	A and B	Neither	AB	O, A, B, AB	I^AI^B
O	45	Neither	Anti-A and anti-B	O, A, B, AB	O	ii

Fig. 30.16 The Landsteiner (ABO) blood groups and the genotypes that give rise to each.

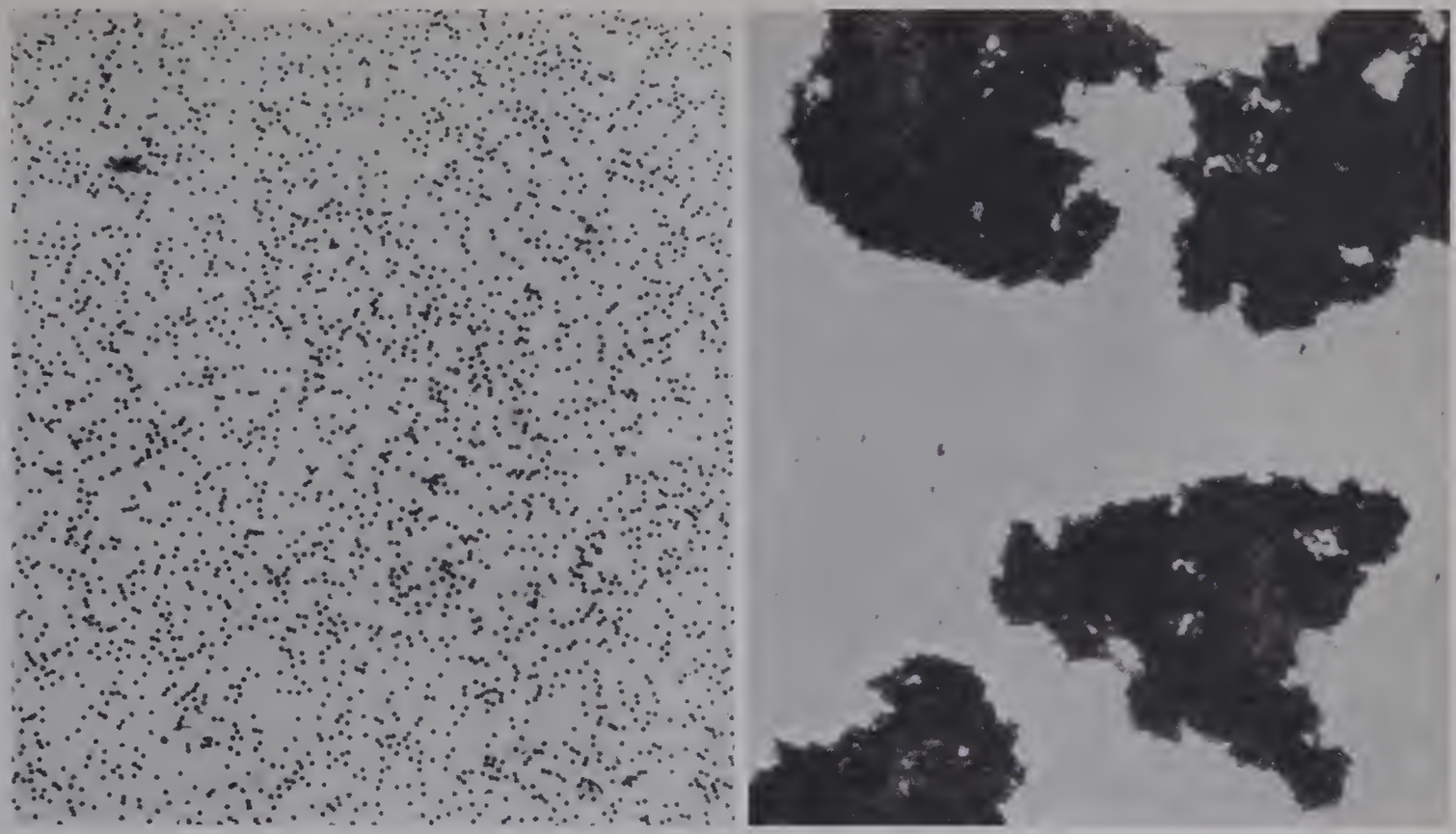


Fig. 30.17 Red blood cells before (left) and after (right) adding serum containing anti-A antibodies. The agglutination reaction indicates the presence of the A antigen on the cells.

blood types can and cannot be safely transfused. The basic principle to be observed is that the blood introduced into the patient's body must not contain RBC's which he can clump. It is not too serious if the introduced blood contains antibodies against his own RBC's because the antibodies will quickly be diluted by his own plasma. Hence, type O blood has been called the "universal donor" because O red cells cannot be clumped and the antibodies will be quickly diluted by the recipient's plasma. Similarly, AB blood is called the "universal recipient" because it contains no antibodies to clump RBC's introduced into it. In actual practice, however, doctors prefer to match blood types exactly when carrying out transfusions.

The ABO system is controlled by a single gene locus

The Landsteiner blood types are inherited. The type is controlled by two alleles, one inherited from each parent. However, there are three alleles, L^A, L^B, and l, present in the population as a whole. Figure 30.16 shows the genotypes that produce each of the blood types. For example, persons with either genotype L^AL^A or L^Al will have A antigens on their RBC's and hence type A blood. Only the genotype L^AL^B produces both A and B antigens and hence blood type AB.

You may well wonder why each person has antibodies against those red cell antigens he lacks. The answer is not entirely certain. Perhaps we are all exposed to substances (in our food, from infections, or from bacteria living in our intestine) that have antigenic determinants similar to A and

B. We would proceed to synthesize antibodies against them if they did not resemble "self," but not if they did. Certainly materials carrying A-like or B-like antigenic determinants turn up from time to time, e.g. as contaminants in vaccines.

Blood transfusions are a special, limited example of tissue transplantation. After three to four weeks the transfused cells have been eliminated from the recipient. But what about transplants, e.g. kidney or liver, that we would hope would survive in their new host indefinitely? In 1956, one kidney was transplanted from a healthy young woman to her ailing *identical* twin. The recipient's life was saved and today both women continue in excellent health. However, when kidney transplants have been made between people other than identical twins, a long-term successful outcome has not been so easy (Section 15.8).

Graft rejection

If we remove a piece of skin from a mouse of one strain and fit it into a site prepared for it on a mouse of another strain, the graft at first does very well. Blood vessels of the host grow into it, and it functions normally. Some 10–14 days after the operation, however, matters take a turn for the worse. The blood supply to the graft breaks down and quickly the graft itself degenerates. Finally, it is sloughed off like an old scab.

The second-set reaction

A clue to the nature of this rejection phenomenon can be gained by trying the experiment again. If we replace the graft with skin from a mouse of a *third* strain, the story repeats itself. But if we try again with skin from the *original* donor strain, the graft may not even last long enough to acquire a blood supply. It is rejected in a much shorter period (5–6 days). This "second-set" phenomenon appears to be the equivalent of the secondary, "memory" response we discussed earlier.

Graft rejection is mediated by specifically sensitized cells

The rejection of a graft is not usually accompanied by any striking appearance of new antibodies in the blood. Instead, it is antigen-sensitive ("killer") cells that play the major role in the rejection process. These cells (lymphocytes) recognize the foreign graft and, by mobilizing other cells in the circulatory system (chiefly monocytes), proceed to kill the cells of the graft.

The lymphocytes that are responsible for graft rejection are not the same as those that serve as the precursors of plasma cells. In order to reject a graft, a mouse must have had a functioning thymus gland at the time its immune machinery was being set up (close to the time of birth). However, the ability of the mouse to secrete *antibodies* in response to injections of Type III pneumococcal polysaccharide is *not* dependent on the thymus. Thus there appear to be two functional divisions of the immune system. One, which is responsible for cell-mediated immune reactions like graft rejection, is dependent on the thymus. The other, which is responsible for the secretion of circulating antibodies, is not.

T cells and B cells: two branches of the immune system

The lymphocytes in each division are formed (like the other blood cells) in the bone marrow. Lymphocytes that will function in cell-mediated immunity, i.e. potential "killer" cells, must then migrate to the thymus before they are able to do their job. Once "processed" by the thymus, these cells are called **T cells.** The lymphocytes responsible for the secretion of

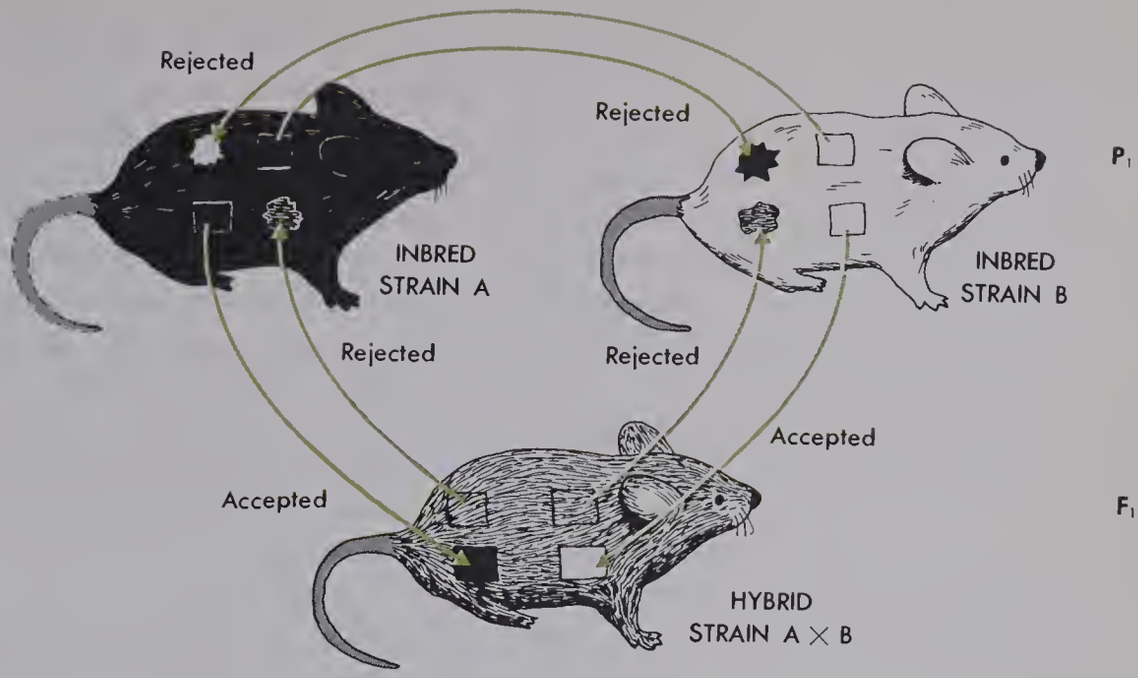


Fig. 30.18 Fate of skin grafts between two inbred strains of mice (A and B) and their F_1 hybrid offspring. The parental types reject each other's skin and also skin from their offspring because in each case it contains "foreign" antigens. The hybrids, however, will accept skin grafts from either parental strain because they have inherited the genes, and thus the antigens, of both. The members of an inbred mouse strain are essentially homozygous and genetically identical and thus, like identical twins, accept grafts from one another.

antibodies are not processed by the thymus. They are called **B cells.** Although there are several ways of discriminating between, and even separating, T cells and B cells, these lymphocytes cannot be visually distinguished under the light microscope.

Our understanding of the graft rejection process is increased by examining the circumstances under which it does *not* occur. First, as we have seen, grafts between genetically identical individuals are not rejected. This can be observed in human identical twins and also in strains of mice that have been inbred for so long that all individuals are virtually identical even though they arise from separate eggs. (Even among such mice, rejection occurs when male skin, *XY,* is grafted onto female, *XX,* but not the reverse. Because of its *Y* chromosome, male skin has genes not possessed by female skin. With this exception, these creatures are essentially homozygous and, thus, the females have no genes foreign to the males.)

Inbred mice are like identical twins

If we breed a mouse of inbred strain A with one of inbred strain B, the F_1 offspring accept grafts from each other and *also* from either of the parental strains (Fig. 30.18). However, neither parental strain will accept an F_1 graft. These findings suggest that whenever genetic differences exist, antigenic differences do also. In this light, we can view the immune system as a device which recognizes the uniqueness of the individual and works to keep it that way.

The immune system recognizes the uniqueness of the individual

What can we do for humans who desperately need a new kidney but have no identical twin to whom they can turn? Two approaches have proved sufficiently workable that kidney transplants now restore thousands to substantial periods of good health. One is tissue typing. Matching blood by ABO (and Rh) blood types permits successful transfusions even though many other red cell antigens exist. While the A and B antigens can provoke serious reactions, the other antigens (e.g. MN) are so weakly antigenic that no problems arise from mismatches. In a similar way, the antigens which the immune system recognizes on the surface of the cells of transplanted organs can be characterized as strong or weak. The rejection mechanism works far more powerfully against the first than against the second. Although a perfect match of all antigens will probably only be possible between identical twins, reasonably good matches—at least with respect to the strong antigens—can often be made, especially between members of the same family.

Tissue typing

Those rare infants born without an immune system, mentioned in Section 30.3, can be *given* one by injections of bone marrow (the source of all the white blood cells) from a healthy donor, usually a brother or a sister. In this case, you might predict that tissue typing would be unnecessary, since the recipients have no mechanism to destroy the transplanted tissue. Actually, tissue typing is just as important in this situation. If strong antigenic differences exist between host and transplant, the *transplant* detects them and mounts an immune response against the *host.* This "graft versus host" reaction is invariably fatal. Although the converse of the usual rejection problem, it illustrates once again the fundamental role that the immune mechanism plays in recognizing "non-self" and destroying it.

Graft vs. host reaction

A second way in which the graft rejection mechanism can be minimized is to treat the recipient with drugs that inhibit the immune response. Because of the need for rapid cell proliferation during an immune response, any drug which inhibits mitosis suppresses the response. For reasons that are not entirely clear, the glucocorticoids (see Section 25.10) are also potent immunosuppressives.

Immunosuppression

While the use of immunosuppressives has been indispensable in enabling transplant recipients to keep their new kidney or heart for substantial periods of time, use of these drugs creates new problems. The drugs now available are not in any sense selective in their action. The more effective they are at suppressing graft rejection, the more effective they are at suppressing the immune response to invading bacteria, fungi, and viruses. While antibiotics and other drugs can help with bacterial and fungal infections, viral infections may become life-threatening.

30.9 CANCER AND IMMUNE SURVEILLANCE

Another complication that turns up in transplant recipients is a disproportionate incidence of cancer. On occasion the cancerous cells are of donor origin, having been inadvertently transplanted along with the organ. The cure in these cases is simple: stop the use of immunosuppressant drugs.

The host's rejection mechanism then promptly destroys the cancerous cells. (The transplanted organ is rejected as well.)

Immune deficiency and cancer

Most of the time, however, the cancer is of host origin. Why should transplant recipients have an increased risk of developing cancer? Could it be related to their immunosuppressed status? This possibility gains plausibility when we realize that there are a number of different hereditary diseases characterized by a poor immune response which are also associated with a disproportionately high incidence of cancer. Perhaps, then, one of the functions of the immune mechanism is to destroy cancerous cells. If so, we would expect that such cells would be recognized by the immune system as "foreign" even though they had *arisen* from "self." Is there any evidence that cancer cells bear antigenic determinants not found on the normal cells of the host?

Cancer cells are "nonself"

The availability of inbred mouse strains and chemical agents which induce cancers in them provides the tools with which to test this idea. We have seen that a mouse will accept as "self" a skin graft from another mouse of the same inbred strain. Will it also accept as "self" a tumor from such an animal? The answer is a qualified no. A transplanted tumor may grow so rapidly that it kills the host before the host can mount an effective immunological attack. Nevertheless, the machinery is there. If such a transplanted tumor is removed before it kills the host, or if the host is first injected with dead tumor cells, the host has a chance to get ready for a "second-set" reaction. Then, when the tumor is reimplanted, it is quickly rejected. The ability of the recipient to recognize tumor cells of its strain as foreign while it accepts normal tissues from that strain as "self" demonstrates that these cancers do bear *new* antigenic determinants.

Many tumors in experimental animals and some, perhaps, in humans are caused by viruses. In these cases, also, the cancerous cells bear antigenic determinants on their surface that are foreign to the host. Known cancer-causing agents (e.g. radiation, many chemicals, many viruses) are distributed widely through our environment. Remembering that cancers probably start as a single aberrant cell, we may well suspect that one of the functions of the immune mechanism is to recognize these aberrant cells and their progeny as foreign and destroy them before they have time to grow into a detectable tumor. Such a function has been described as **immune surveillance.**

If such is the case, why should we ever succumb to cancers? There are several possibilities. The incidence of cancer (after a small peak in childhood) increases with advancing age. The effectiveness of the immune mechanism (as with so much of the body's machinery—see Section 23.3) decreases with advancing years. Furthermore, many of the chemicals known to induce cancer (e.g. hydrocarbons such as benzopyrene—see Section 12.10) are also immunosuppressives. The same is true of some of the cancer-causing viruses. Perhaps part of the very basis of the carcinogenic activity of these agents is their effectiveness in suppressing immune surveillance.

Many carcinogens are immunosuppressives

The immune mechanism may itself be responsible for the breakdown of immune surveillance and the success of a tumor in gaining the upper hand. Tumor rejection, like graft rejection, is primarily a matter of specific "killer" lymphocytes (T cells) leading an attack on the foreign cells. Antibodies circulating in the bloodstream appear not to be much help in the process and may, indeed, be detrimental. There is much evidence accumulating that some type(s) of circulating antibodies may *inhibit* the rejection phenomenon by combining with determinants on the "foreign" cells. In so doing, they cover or mask these determinants so that they are not able to trigger the cell-mediated rejection process. In this way, a state of tolerance to the tumor may develop.

Antibodies may sometimes protect cells from attack

30.10 AUTO-IMMUNITY

Why do we not make antibodies against our own tissue components? Or, to put the question another way, what is the mechanism of self-tolerance?

The forbidden clone hypothesis

A theory of self-tolerance that has long been popular is that of "forbidden" clones. Forbidden clones are those clones of lymphocytes directed against self-components. According to this theory, they are destroyed, in some manner, by the time the immune machinery is set up shortly after birth. This theory explains nicely the fact that, as we have noted earlier, antigens introduced into the animal prior or close to birth are henceforth accepted as self. Presumably their presence had established additional forbidden clones, which were then destroyed.

There is now a wealth of evidence, both in humans and in laboratory animals, that we *can* under certain circumstances manufacture antibodies and/or sensitized ("killer") lymphocytes against self-components. The serum from patients with multiple sclerosis contains antibodies which lead to the destruction of the myelin sheath of nerve cells, even in tissue culture. Physical damage to the choroid layer of one eye may later lead to immunological damage to the choroid layer of the second eye. Some people develop both antibodies and sensitized lymphocytes directed against the proteins and cells of the thyroid gland. In these examples, as well as a number of others, the disease process appears to stem from the immunological mechanism attacking self-components. Such disorders are now called **auto-immune** diseases. Their existence does not necessarily rule out the forbidden-clone hypothesis. In each of the examples cited, the tissue under attack is not normally exposed to the system of lymph nodes where the immune response occurs. The axons of the spinal cord, for example, are bathed in cerebrospinal fluid, not blood, and there is no lymphatic drainage. One can argue, therefore, that antigenic determinants in these "privileged" sites were not exposed to the immune system at the time that the forbidden clones were being eliminated.

Auto-immune diseases

An all-too-common consequence of rheumatic fever is damage to the heart valves. In many ways, this too resembles an auto-immune disease. Antibodies are present which react not only with the cell walls of the streptococci that cause rheumatic fever, but also with components in the

heart muscle. But here, too, it may be that active clones are not *primarily* directed against self-components but rather against the antigenically similar streptococcal determinants.

Looking for forbidden clones in the tetraparental mouse

A better test of the forbidden-clone hypothesis is to examine a remarkable animal we met in an earlier chapter (see Section 21.8): the tetraparental mouse. Here is a creature constructed from two antigenically different kinds of cells. A-strain and C-strain mice reject each other's grafts. What about a mouse that is a mixture of A cells and C cells? Will the A lymphocytes mount an attack against C cells and C lymphocytes attack A cells? This does not happen; the mice—like the cattle twins discussed earlier—tolerate the mixture of cells with no signs of autoimmunity. Does this mean, then, that *two* sets of forbidden clones were eliminated during the development of the mouse? The answer is no. Spleen cells removed from a tetraparental mouse, and placed in a tissue culture that contains *no serum* from the mouse, readily attack both A-strain and C-strain cells. Adding the tetraparental serum to the cultures protects the cells from attack. The situation is thus similar to that we found with many cancerous tumors: the presence in the blood of substances (presumably antibody molecules) that, instead of attacking cells, protect them from attack.

Clearly there are no forbidden clones in tetraparental mice. Are there forbidden clones in biparental vertebrates? Or is self-tolerance instead a matter of developing special antibodies that protect our own components from attack by those lymphocytes sensitized to their antigenic determinants? Only more research will tell us the answer.

30.11 CODA

For many decades the study of the immune response centered upon its role in protecting vertebrates (and immunity *does* appear to be exclusively a vertebrate attribute) from infectious disease agents. But now we can see the immune response in broader perspective. The immune mechanism enables the vertebrate body to synthesize a very large (*how* large is as yet unknown) number of different kinds of protein molecules. Among this diverse collection will be found a few that are capable of physically combining with, as far as we can tell, any given three-dimensional molecular configuration (determinant) that can be devised. This machinery does not ordinarily lead to destruction of macromolecules that are normal constituents of the host. The machinery *does* act to inactivate and destroy macromolecules that enter the internal environment from the outside world. In so doing, it protects us from infectious agents such as viruses and bacteria. It may also produce unpleasant symptoms of allergy when noninfectious agents (e.g. ragweed pollen) gain entrance. It also works to destroy the surgeon's hopeful efforts to replace damaged organs. And, in all likelihood, the immune system stands ready to destroy aberrant (cancerous) cells that arise in the body. In carrying out these actions, the system learns

from its experience and usually develops an enhanced capacity to react to subsequent encounters with the same configuration.

The immune system works to preserve the integrity of the body

The immune mechanism thus acts to guard the body against invaders from without and the cancerous cells that threaten to invade us from within. In the words of F. M. Burnet, one of the great immunologists of the twentieth century, the immune response works to preserve "the integrity of the body."

EXERCISES AND PROBLEMS

1. Explain why the antibody-containing cells shown in Fig. 30.14 must first be treated with antigen before they can be made visible by treatment with fluorescent antibodies.
2. Why do you suppose that pretreatment with antibodies directed against mouse antibodies (hence, anti-antibodies) prevents the cells shown in Fig. 30.11 from sticking to the antigen-coated fibers (and to antigen-coated red cells—Fig. 30.12)?

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A 100-million-year-old ant *(Sphecomyrma freyi)* fossilized in amber. This animal has a number of wasplike features and represents a "missing link" in the evolution of ants from wasps. The specimen was found in New Jersey. (Courtesy of Frank M. Carpenter, Harvard University.)

Part VI EVOLUTION



31 EVOLUTION: THE EVIDENCE

The Evidence from Paleontology

The Evidence from Comparative Anatomy

The Evidence from Embryology

The Evidence from Comparative Biochemistry

The Evidence from Chromosome Structure

The Evidence from Protective Resemblance

The Evidence from Geographical Distribution

The Evidence from Domestication

Darwin's Origin of Species

In 1859, the British naturalist Charles Darwin published his *Origin of Species.* It has been said that this book ranks second only to the *Holy Bible* in its impact on man's thinking. What did it say that made it so influential?

First, the *Origin of Species* said that all living things on earth are here as a result of descent, with modification, from a common ancestor. This is the theory of **evolution.** Expressed another way, it tells us that species are not fixed, unchanging things but, on the contrary, have, through a process of gradual change, evolved from preexisting, different species. The theory implies, too, that all species are cousins, that is, any two species on earth have shared a common ancestor at some point in their history. This theory of evolution directly contradicts the still widely accepted idea that species are unchangeable, each species having been placed on earth in its present form.

Second, Darwin's *Origin of Species* presented a large number of facts which Darwin felt could best be explained by a theory of evolution and could not be adequately explained by a theory of special creation. In this chapter, we shall examine some of these facts along with additional evidence that has been discovered since Darwin's time.

Finally, Darwin proposed a mechanism to explain how evolutionary change takes place. This theory, the theory of **natural selection,** is the cornerstone of the *Origin of Species.* The idea of evolutionary change is very old. Evidence to support it had been presented before Darwin's time. It was Darwin, however, who built a virtually airtight case for the idea and then proposed the theory of natural selection to explain how evolution works. This theory and the clarification and enlargement it has received at the hands of later workers will be discussed in Chapter 32.

The idea of evolution provides a plausible explanation for a host of otherwise hard-to-explain facts. Let us now turn our attention to these.

31.1 THE EVIDENCE FROM PALEONTOLOGY

Paleontology is the study of **fossils.** We can define a fossil as any sort of remains of a once-living organism. A variety of kinds of fossils are found. Figure 31.1 shows the footprints left by dinosaurs some 120 million years ago as they walked along the bed of a stream in the region that is now central Texas.

Kinds of fossils

Under special circumstances, the **entire body** of an organism may be preserved after death. Insects, trapped in the sticky pitch of conifers growing along the Baltic Coast over 30 million years ago, can now be studied entombed in amber as easily as if they had just died (Fig. 31.2). Perhaps you have heard stories of the frozen woolly mammoths found in Siberia in the early years of this century. The meat of these animals was sufficiently well-preserved to be fed to dogs after thousands of years in nature's deep freeze.

Such total preservation of dead organisms is very rare. Usually, the soft portions of the body are quickly destroyed after death by scavengers or decomposed by decay bacteria. **Hard parts,** such as bones or shells, are



Fig. 31.1 Dinosaur footprints in limestone near the town of Glen Rose, Texas. The tracks on the right were made by a large dinosaur walking on all four legs. The tracks on the left were made by a smaller, bipedal dinosaur, perhaps a predacious species. The tracks were formed during the Cretaceous period some 120 million years ago. (Courtesy of Roland T. Bird.)

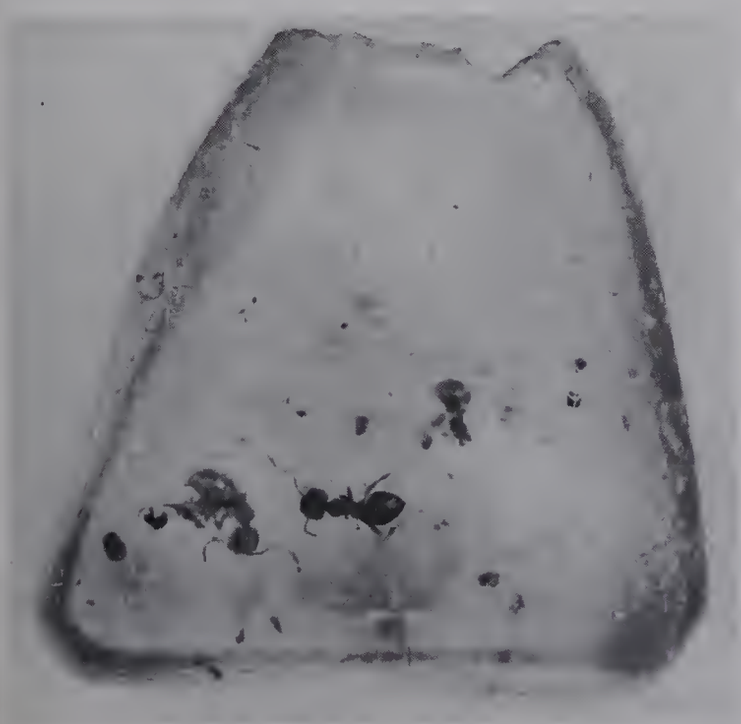


Fig. 31.2 Ants fossilized in a piece of amber. These ants are only one-third as old as the specimen shown at the beginning of Part VI.



Fig. 31.3 Partially exposed dinosaur bones in Wyoming. (Courtesy of the American Museum of Natural History.)

more resistant to such destruction and hence more likely to be fossilized. If surrounded by sediments of clay or sand, they may yield easily recognizable fossils 500 million years later, long after the enclosing sediments have turned to rock such as shale or sandstone (Fig. 31.3). These fossils may even retain traces of organic matter for surprisingly long periods. Amino acids and small peptides have been recovered from some that are over 300 million years old.

Another common fossil is the **petrifaction.** This is a copy in stone of some plant or animal part. As the original remains disintegrated, they were replaced bit by bit with mineral deposits. This process can proceed so slowly that the original specimen is reproduced in all its detail. Figure 31.4 shows the clearly preserved annual rings in a piece of petrified wood. None of the original material is present in this specimen. It is not made of cellulose but of silica. Nevertheless, the faithfulness of the copy makes the fossil as useful as the actual specimen would be.

We know that fossils have aroused the curiosity of man at least since the time of the ancient Greeks. With rare exceptions, fossils are not the remains of organisms still found living on the earth. How, then, can we explain their existence? A series of special creations followed by worldwide catastrophic extinctions has sometimes been given as the explanation. The theory of evolution provides a more satisfying answer, however.

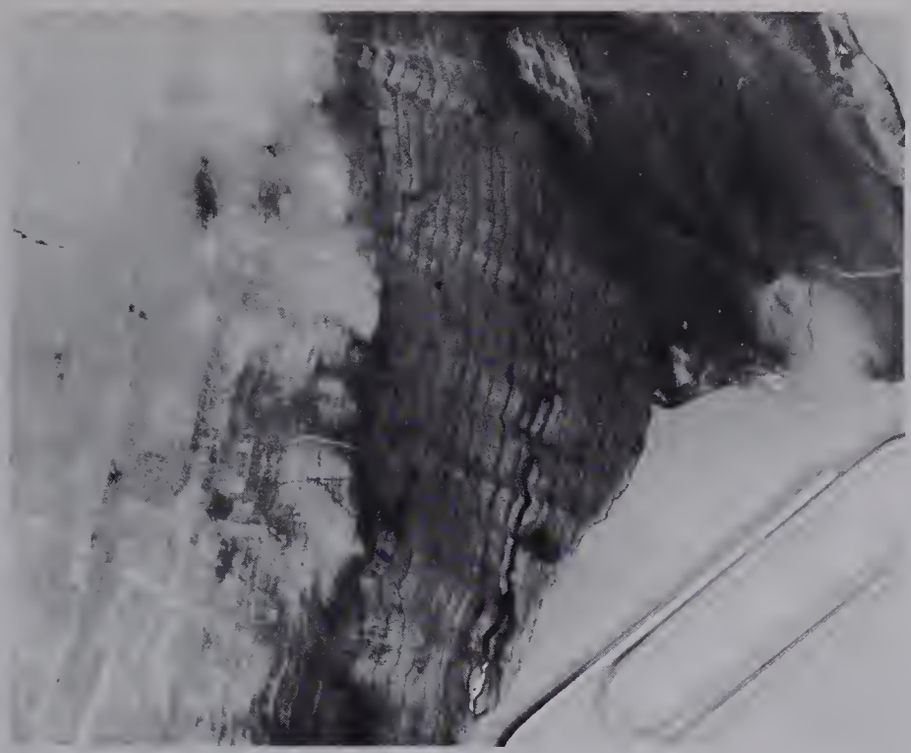


Fig. 31.4 A piece of petrified cypress wood. Although all organic matter has been replaced by silica, the pattern of annual rings stands out clearly.

The fossil record reveals a general increase through the years of the diversity and complexity of life

The idea that all organisms alive today share a common ancestry at some period in history implies that there were fewer kinds of living things in the past and that these were less complex. This describes the fossil record very well. In descending into the Grand Canyon (Fig. 31.5), one passes layer after layer of sedimentary rock, the deeper layers being the older ones. As one proceeds downward, the number of different kinds of fossils decreases. Furthermore, the complexity of the organisms represented in the deeper layers is less than in the upper layers. Fossil reptiles appear late in the geological record while fossil worms appear very early.

It must be noted that one never finds an unbroken fossil history in one location. Geological upheavals of the land (which, after all, is the only way fossils of marine organisms could ever be exposed to our view) are always followed by erosion and hence erasure of part of the fossil record. Sometimes sedimentary rock of an earlier time may become thrust over younger rock. Fortunately, geologists can easily detect these "unconformities."

The problem of "missing links"

Many of Darwin's critics cited the failure of paleontologists to find "missing links" as a serious weakness of the idea that present forms have evolved from forms now known only as fossils. The argument has steadily lost effectiveness as more and more "missing links" have been found. If a biologist should try to describe an animal intermediate between the birds of today and the reptiles from which we believe they evolved, he would almost perfectly describe *Archeopteryx,* one of the many no-longer-missing links (Fig. 37.13).

Gaps in the fossil record are still quite noticeable among the soft-bodied animals and among the prehumans. This is not surprising when you consider the remote chances of either type of organism's becoming fossilized in the first place. Soft-bodied animals are likely to decay too quickly



Fig. 31.5 Grand Canyon as seen from the south rim. The various layers of sedimentary rock, spanning much of the Paleozoic era, are clearly visible. (Courtesy of Josef Muench.)

and terrestrial ones (especially intelligent primates) are not likely to die where their remains will be quickly protected by enclosing sediments.

Then, too, we must remember that we can never expect to find more than fragments of the fossil record. Most of the fossils ever formed are still imprisoned within mountain ranges, under the earth and oceans, or have been destroyed by subsequent erosion and other geological disturbances.

The fossil record is dominated by organisms that were abundant and widespread

Perhaps the greatest obstacle to finding missing links is that the evolution of new species of plants and animals seems, in general, to have occurred in small populations of poorly specialized forms. The fossil record is, however, filled chiefly with the remains of "climax" groups, large populations of highly specialized organisms which flourished for a period only to become extinct when conditions on earth changed.

Though we may never be able to trace the evolution of all living forms through the fossils of their ancestors, the presence and distribution of fossils already discovered provide us with some of the most direct evidence of the theory of evolution.

31.2 THE EVIDENCE FROM COMPARATIVE ANATOMY

Homologous organs are fundamentally alike in structure, embryonic development, and relationship to other organs

In comparing the anatomy of one mammal with another, one cannot help being struck by the many cases in which certain parts of the body are built to the same basic plan in each specimen. This might not seem surprising if the similarly constructed parts were used in similar ways. One could argue that there was only one best way to construct the organ in question and that the Creator used it. However, these organs may actually be used in a variety of ways. Figure 31.6 illustrates the organization of bones in the forelimbs of the human, a whale, and a bat. Although these forelimbs are used for such diverse activities as lifting, swimming, and flying, the same basic structural plan is evident in all of them. Organs which have the same basic structure, the same relationship to other organs, and (as it turns out) the same type of embryonic development are said to be **homologous.**

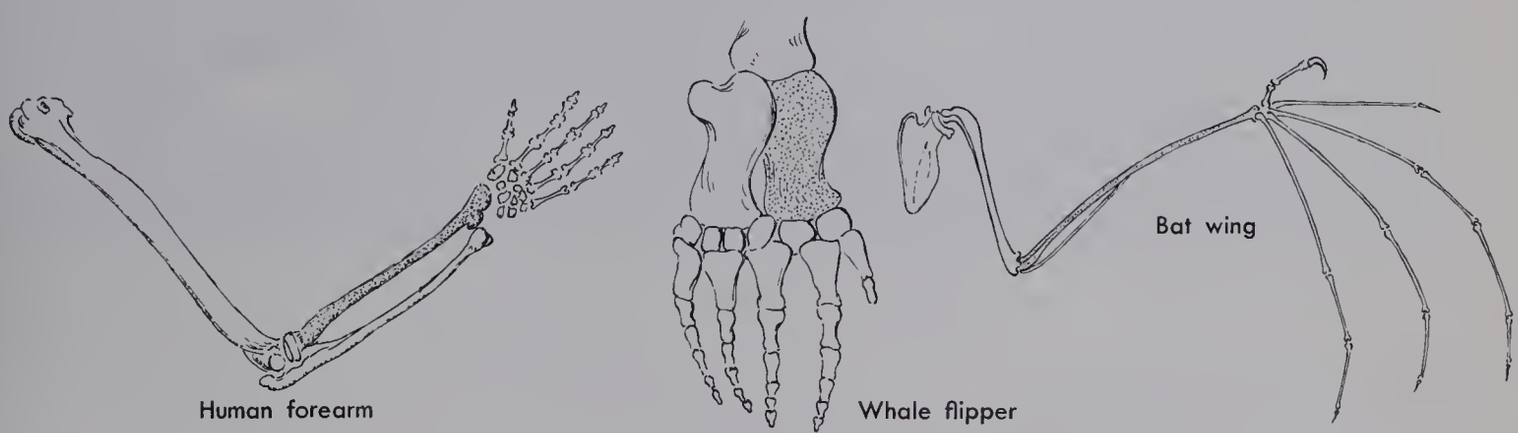
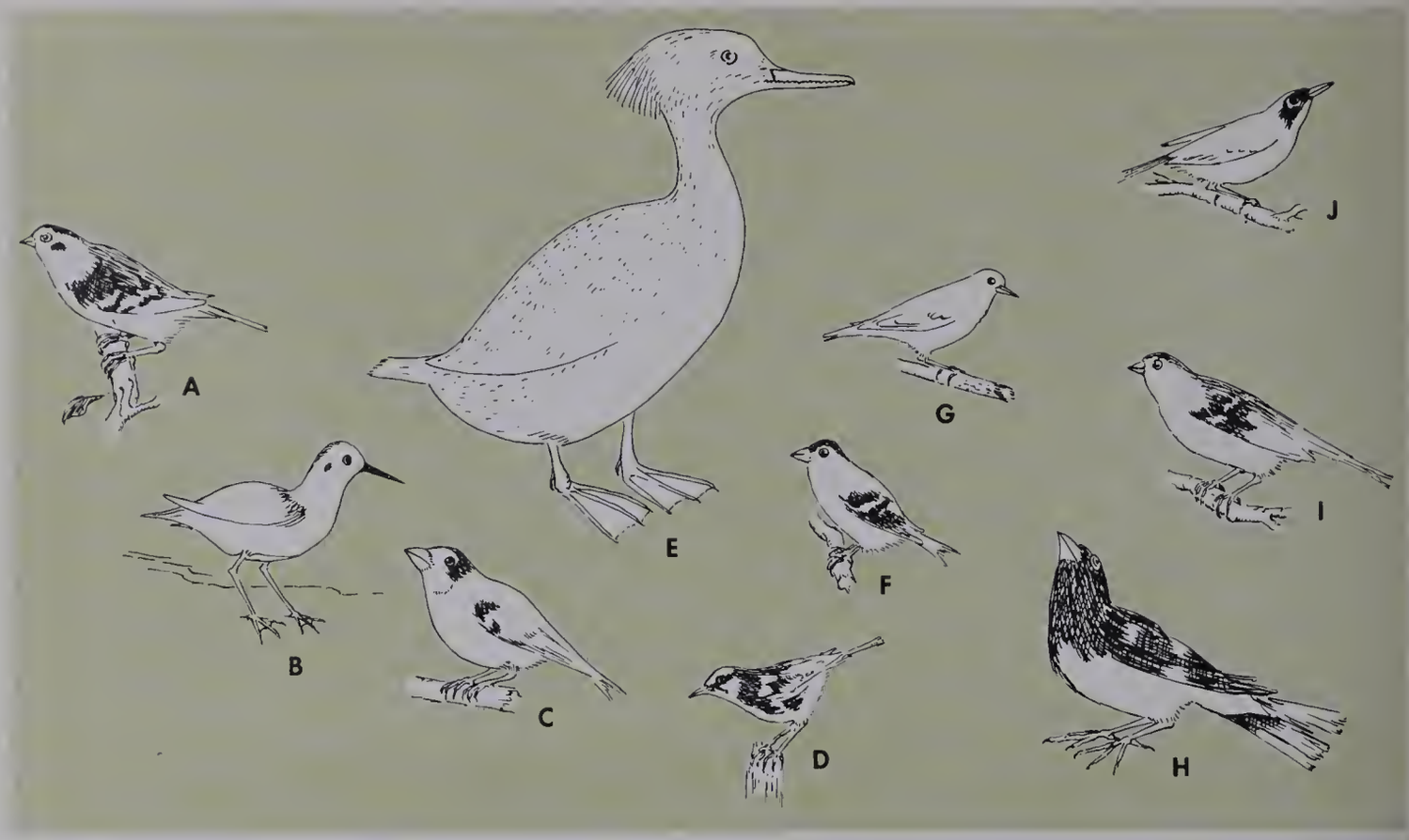


Fig. 31.6 Three vertebrate forelimbs; a study in homologous organs. In each case the bone that is stippled is the radius. The forelimbs are not drawn to the same scale.

It hardly seems reasonable that a single pattern of bones represents the best possible structure to accomplish the varied tasks to which these mammalian forelimbs are put. However, if we interpret the persistence of the basic pattern as evidence of inheritance from a common ancestor, we see that the various modifications are simply adaptations of the plan to the special needs of the organism.

The various mammalian forelimbs constitute just one example of homologous organs. Both the animal and plant kingdoms contain a large catalogue of such structures. This is hardly surprising in view of our belief that all organisms have shared a common ancestor at some time in their evolutionary history. Presumably, the more recently two species have shared an ancestor, the more homologous organs they will have in common. Homologies in more distantly related species may be harder to determine, although fossil evidence is often a great help in this.

We *classify* organisms on the basis of the number of shared homologies. The greater the number of these, the smaller the taxonomic unit in



Distribution of Eight Homologous Structures

SPECIES	A	B	C	D	E	F	G	H	I	J
1. Feathers	+	+	+	+	+	+	+	+	+	+
2. Serrated bill					+					
3. Partial webbing between toes		+								
4. Perching feet	+		+	+		+	+	+	+	+
5. Slender bill				+			+			+
6. Short, stout bill	+		+			+		+	+	
7. Yellow plumage on top of head				+			+			
8. Reddish-brown plumage on back	+								+	

Groupings Made on the Basis of These Structures

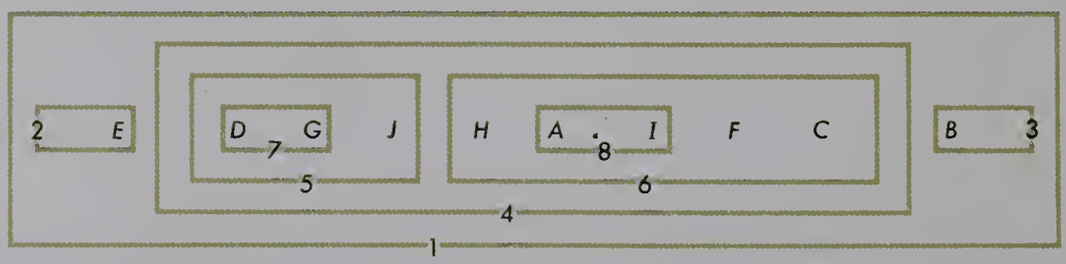


Fig. 31.7 Ten birds; a study in the techniques of classification.

Classifying organisms by number of shared homologies

which we place the organisms. Figure 31.7 shows ten organisms. It is perfectly clear that all ten are animals and even that all ten are birds. But how should they be classified with respect to one another? Closer inspection reveals that some of the birds (*A* and *I*, for example) are quite similar in appearance. Perhaps, then, these should be placed together in one category. Another category might include *D*, *G*, and *J*, which show similarities of bill shape, size, and general body contour. *C*, *F*, and *H* seem to fall into still a third group, while *B* and *E* seem quite different in appearance. In fact, our first three groups seem to have more features in common with each other than they do with either *B* or *E*. Perhaps these three subgroups should be placed together in a larger group which excludes *B* and *E*.

This, then, is the basic process involved in classification. Of course, we have been somewhat limited in our efforts here by being restricted to a view of external anatomy. Were a knowledge of all homologous features available to us, we could tabulate the distribution of each of these throughout the entire group. Those birds sharing the largest number of homologous features would be grouped together. This reflects our belief that they are most closely related, in other words, have most recently descended from a common ancestor. These first groups would then be organized into larger groups on the basis of all their members' sharing a smaller number of homologous features. We assume that the members of these larger groups are more distantly related than the members of the smaller groups. The common ancestor from which they are descended existed in a more remote time.

The Classification of Man

Kingdom	— Animal
Phylum	— Chordata
Subphylum	— Vertebrata
Class	— Mammalia
Order	— Primates
Family	— Hominidae
Genus	— *Homo*
Species	— *Homo sapiens*

Fig. 31.8 The major taxonomic categories as they are used in the classification of man. Plant taxonomists use the term *division* instead of *phylum*.

In the case of our birds, each one represents a single kind, or **species,** of bird. This is the fundamental unit of classification. The groups in which we place the various species are called genera (singular, **genus**). The two sparrows, *A* and *I*, are classified in the genus *Spizella*. Related genera are, in turn, placed in **families.** Related families make up **orders,** and orders are grouped together in **classes** (in this case, Aves, or birds). Similar classes are placed in a given **phylum** and all related phyla make up a kingdom (Fig. 31.8).

In addition to these major groups, taxonomists often find it convenient to establish others: phyla may be divided into subphyla, and superas well as subclasses and -families are also formed in some cases. The currently accepted classification of our ten birds is shown in Fig. 31.9.

Evolutionary implications of modern taxonomy

If, as we hope, our classification scheme reflects degree of kinship, then we should be able to set up a family tree showing the evolutionary history of the group (Fig. 31.9). The species are all placed at the tips of the branches, and the common ancestors at the forks. It is important to remember two things when dealing with such a family tree. First, if the genealogy is correct, all the species in any one group, no matter what its rank, have shared a common ancestor among themselves more recently than they have with species in any other group. Second, no living organism is the ancestor of other organisms. This seems obvious, but many biology students persist in thinking that the more primitive organisms living today are the ancestors of the more complex organisms. This is not true, although

Class Aves—birds
Subclass Neornithes—modern birds
Superorder Neognathae—flying birds
Order Anseriformes—flat-billed swimmers
Family Anatidae—swans, geese, ducks
Subfamily Mergansers (*E*)
Order Charadriiformes—shore birds
Family Scolopacidae
Genus *Ereunetes* (*B*)—sandpiper
Order Passeriformes—perching birds
Family Parulidae—warblers
Genus *Dendroica* (*D* and *G*)
Genus *Geothlypis* (*J*)
Family Fringillidae
Genus *Spizella* (*A* and *I*)—sparrows
Genus *Pheucticus* (*H*)—grosbeak
Genus *Carpodacus* (C)—finch
Genus *Spinus* (*F*)—finch



Fig. 31.9 The actual classification of the ten birds shown in Fig. 31.7 and a deduction as to the evolutionary history of these birds.

we may assume that during the course of evolutionary descent from a common ancestor, the more primitive organisms have changed less than the more complex ones. The only direct evidence of common ancestors ("missing links") that we can ever hope to find is their fossilized remains.

This second point is related to a third: the general history of life on earth seems to have been the formation of more and more different kinds of organisms. Each fork in the family tree represents two kinds of organisms *diverging* from one, the common ancestor.

Vestigial organs

One category of homologous organs deserves special mention for providing evidence of evolution. These are homologous organs which in some species have no apparent function. Dissection of a boa constrictor or a whale reveals bones that are thought to be homologous to the hip bones of other vertebrates. No function seems to be accomplished by these structures. If all species have been specially created, it seems like poor designing to include nonfunctional parts. If, on the other hand, we assume that both snakes and whales have evolved from four-legged ancestors, then we can understand why traces of their evolutionary heritage still remain.

Man, too, has similar **vestigial organs.** The fused vertebrae which make up the base of the human spine are interpreted as the vestigial remnants of the tail possessed by our ancestors. In fact, human babies occasionally are born with short tails. These are, however, quickly and easily removed.

31.3 THE EVIDENCE FROM EMBRYOLOGY

Anatomical recapitulation

The embryonic development of all vertebrates shows striking uniformities. This is particularly true during cleavage, morphogenesis, and the early stages of differentiation (Fig. 31.10). These similarities are often cited as

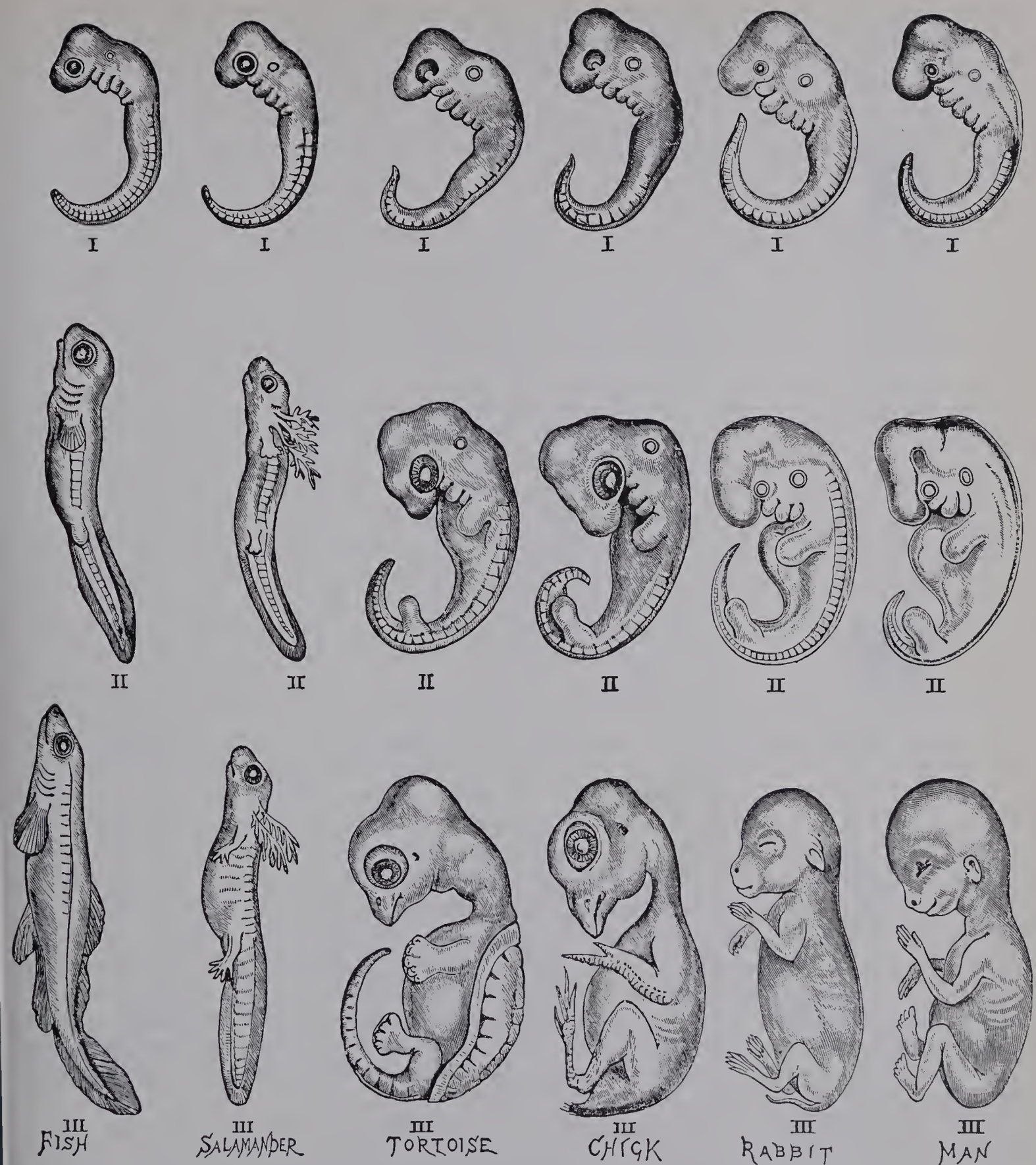


Fig. 31.10 Comparison of vertebrate embryos. (G. J. Romanes, *Darwin and After Darwin,* Open Court Publishing Co.)

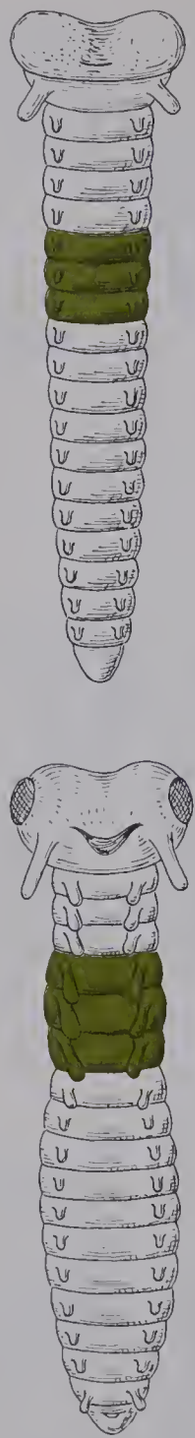


Fig. 31.11 Two stages in the embryonic development of an insect. Although limb buds appear in all segments, as they surely did in the ancestors of the insects, only those in the thorax (color) become legs. The ones in the head develop into mouth parts. The ones in the abdomen disappear.

evidence of an evolutionary relationship among the vertebrates. Of course, one might argue that there is only one effective way that a vertebrate can be constructed from a fertilized egg. There may well be considerable truth in this idea but, on the other hand, it does not adequately explain one aspect of these similarities. This is the fact that some structures appear during the development of the most advanced vertebrates just as they do in the more primitive species, only to disappear or become almost unrecognizably modified in the later stages of development. The month-old human embryo has a series of paired branchial grooves in the neck region (Fig. 31.10). These are matched on the interior by a series of paired gill pouches. This pattern appears not only in man but in the embryonic development of all vetebrates. In the fishes, the pouches and grooves eventually meet and form gill slits, the openings which allow water to pass from the pharynx over the gills and out of the body. In the "higher" vertebrates the grooves and pouches disappear. In man the chief trace of their existence is the Eustachian tube and auditory canal, which (interrupted only by the eardrum) connect the pharynx with the outside of the head. The temporary possession of a tail and a two-chambered heart are other examples of developmental stages through which the human embryo passes. Surely there must be more direct ways to achieve the final adult form. What explanation, then, can we give for these seemingly inefficient procedures? Again evolution provides the clue. We and the other vertebrates continue to pass through many (not all) of the embryonic stages that our ancestors passed through because we have all inherited developmental mechanisms from a common ancestor. We then go on to modify these in ways appropriate to our diverse ways of life. Therefore, it should be no surprise to find that the more distantly related two vertebrates are, the shorter the period during which they pass through similar embryonic stages (Fig. 31.10). Conversely, the more closely related two vertebrates are, the longer their embryonic development proceeds in a parallel fashion.

The idea that our embryonic development repeats that of our ancestors is called the theory of **recapitulation.** This theory is not restricted to the vertebrates. There is some evidence (which we shall examine in Chapter 36) that the ancestors of the insects had a pair of legs on each of their body segments. In this, they resembled today's millipedes. (In fact, the millipedes may well represent a second line of descent from these early forms.) In any case, during the embryonic development of insects, limb buds appear on the abdomen just as they must have in their multi-legged ancestors (Fig. 31.11). But by the time the larva hatches, only the six legs on the thorax remain.

Biochemical as well as anatomical recapitulation occurs. As you learned in Chapter 15, fish excrete a large part of their waste nitrogen as ammonia, while amphibians have the less toxic urea as their chief nitrogenous waste. Actually, the fishlike *tadpole* excretes ammonia until it undergoes metamorphosis into the adult frog. Only then does its chief nitrogenous waste become urea.

Birds go one step further and convert their waste nitrogen compounds into the almost insoluble compound, uric acid. It is interesting to see (Fig. 31.12) that the developing chick embryo does not excrete uric acid from the very first but instead passes through an ammonia-excreting stage followed by a urea-excreting stage. This certainly suggests that the chick is repeating stages in the biochemical development of its ancestors.

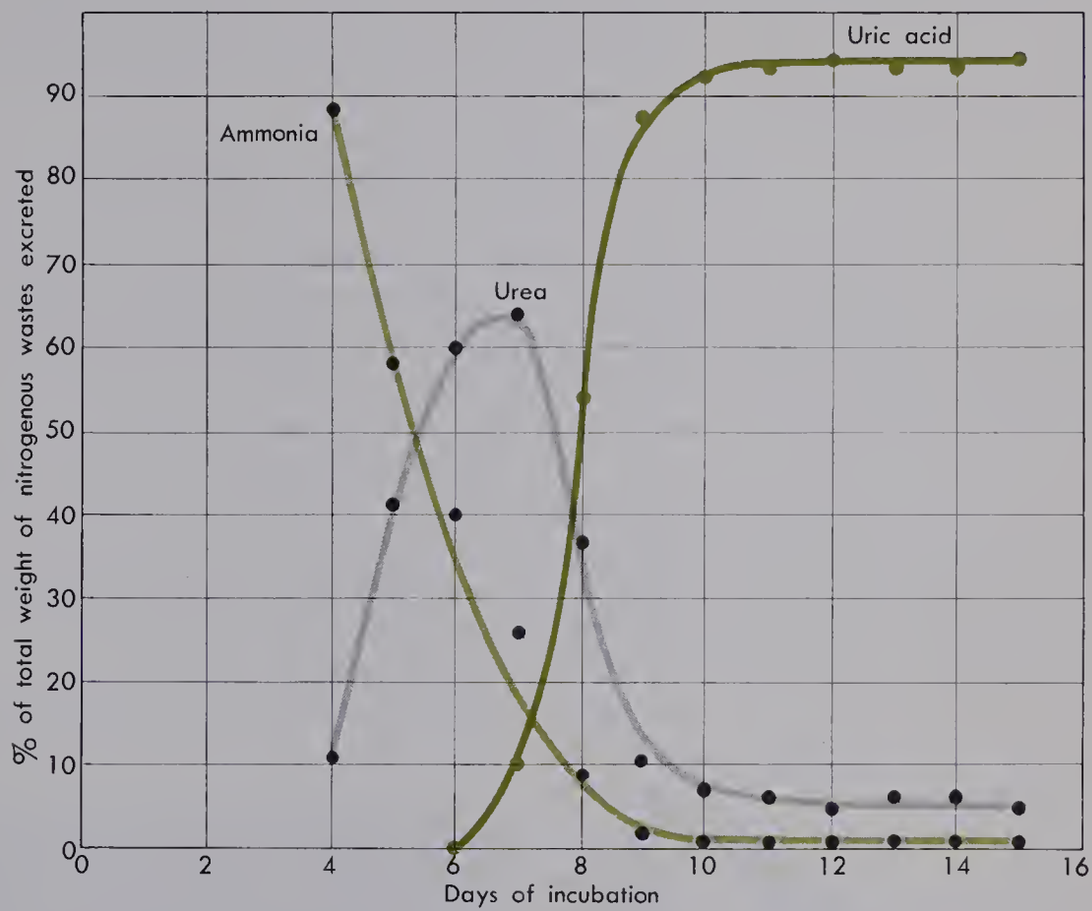


Fig. 31.12 Biochemical recapitulation in the chick embryo.

31.4 THE EVIDENCE FROM COMPARATIVE BIOCHEMISTRY

Just as the study of comparative anatomy has shown the presence of anatomical homologies, so the study of the biochemistry of different organisms has revealed biochemical homologies. In fact, the biochemical similarity of living organisms is one of the most remarkable features of life.

Estimates of kinship can be based on similarities of protein structure

Cytochrome enzymes are found in almost every living organism. One of these, cytochrome *c*, is a polypeptide chain of 104 to 112 amino acids (depending upon the organism in which it is present). In recent years the exact sequence of amino acids in these chains has been determined for the cytochrome *c*'s of such diverse organisms as man, the rabbit, the king penguin, the rattlesnake, the tuna fish, a moth, the red bread mold *Neurospora,* and many others. Although there is considerable variation in the sequences, especially between organisms that we assume are only distantly related, there is a surprising amount of similarity as well. The sequence in man differs from that in the monkey at only one place in the chain.

Cytochrome *c* from the wheat plant differs from man's in 35 of the amino acids. However, another 35 of the amino acids in the chains have proved to be the same in every single species examined. This includes one section of 11 consecutive amino acids (Nos. 70–80) that are common to all organisms known. We have seen how the nucleotide sequence in DNA molecules codes for the amino acid sequence in proteins. The occurrence in such a wide variety of organisms of genes for cytochrome *c*—genes that contain much of the same genetic information—would be virtually inexplicable without a theory of evolution. Surely this phenomenon means that we have all inherited this gene, albeit with an accumulation of mutations, from a common ancestor.

The same argument can be applied to other biochemical similarities among organisms. Studies of the amino acid sequences in mammalian hemoglobins reveal close similarities, especially so among those species thought to be closely related. DNA and RNA are found in every living organism and, so far as we can determine, contain the same hereditary coding mechanism. Furthermore, most vertebrates share the same, or similar, hormones. LTH, for example, is found in vertebrates as diverse as fishes, birds, and mammals, although its function differs in each of these classes (see Section 25.9). Thus we have a parallel on the chemical level of the homologous forelimbs we discussed earlier in the chapter: a hormone inherited from a common ancestor but with its function modified in ways appropriate to the life of each animal.

The remarkable uniformity of biochemical organization that underlies the incredible diversity of living things is difficult to interpret in any other way but an evolutionary one. Presumably these molecules appeared very early in the history of life, and almost all modern forms have inherited the ability to manufacture and use them.

If one injects human serum proteins into a rabbit (a rabbit is simply a convenient animal to use—any other mammal would do), the rabbit manufactures a wide variety of antibody molecules against all the antigenic determinants foreign to it. When rabbit-blood serum containing these antihuman antibodies is mixed in a test tube with human serum, insoluble antigen-antibody complexes form that settle out of solution as a precipitate. The amount of precipitate formed can be easily measured. What makes this reaction interesting so far as our present story is concerned is that these antihuman antibodies will also react with the blood serum of certain other mammals but to a lesser degree; that is, a smaller amount of precipitate is formed. Antihuman antibodies mixed with the serum of man, an ape, an Old World monkey, a New World monkey, and a pig (in each of five separate test tubes) produce a precipitate in each tube. The amount produced decreases, however, from man to pig (Fig. 31.13). As we shall see in Chapter 37, this corresponds closely to the presently accepted degree of kinship between man and these other mammals.

Not only has this method (called comparative serology) corroborated some evolutionary relationships which had already been agreed upon, but it has been very helpful in establishing relationships for which anatomical

Fig. 31.13

Reaction between antihuman antibodies (prepared in rabbit) and serum of various mammals, with human taken as 100%

Man	100%
Chimpanzee	97%
Gorilla	92%
Gibbon	79%
Baboon	75%
Spider monkey*	58%
Lemur	37%
Hedgehog (Insectivora)	17%
Pig	8%

*New World species

evidence had failed to provide clear-cut answers. For many years, the exact position of the horseshoe crab, *Limulus* (Fig. 36.17), among the arthropods was unclear. Now, however, a strong reaction between anti-*Limulus* antibodies and arachnid antigen has caused *Limulus* to be placed in the class Arachnida. Similarly, rabbits, although they show some structural resemblances to rodents, are now placed in an order of their own, the Lagomorpha. Serological tests show that little affinity exists between rabbits and rodents; in fact, rabbits seem to be more closely related to even-toed ungulates like the pig! Whales, too, reveal by serological testing a closer relationship to the even-toed ungulates than any other mammalian order. Even plant proteins have been used as antigens and several evolutionary puzzles have been cleared up by this technique.

31.5 THE EVIDENCE FROM CHROMOSOME STRUCTURE

The differences that distinguish one species from another are, in the last analysis, genetic. Genes are located on chromosomes. If, then, we believe that all species share a common ancestor at some point in time, can we find homologies in chromosome structure just as we do in skeletal structure (Fig. 31.6) and protein structure?

Kinship is reflected in karyotypes

The more closely related two species appear to be on the basis of such criteria as homologous organs, the more similar are their karyotypes. The karyotypes of a chimpanzee and an orangutan are practically indistinguishable and, except for the presence of 48 instead of 46 chromosomes, very similar to that of a human.

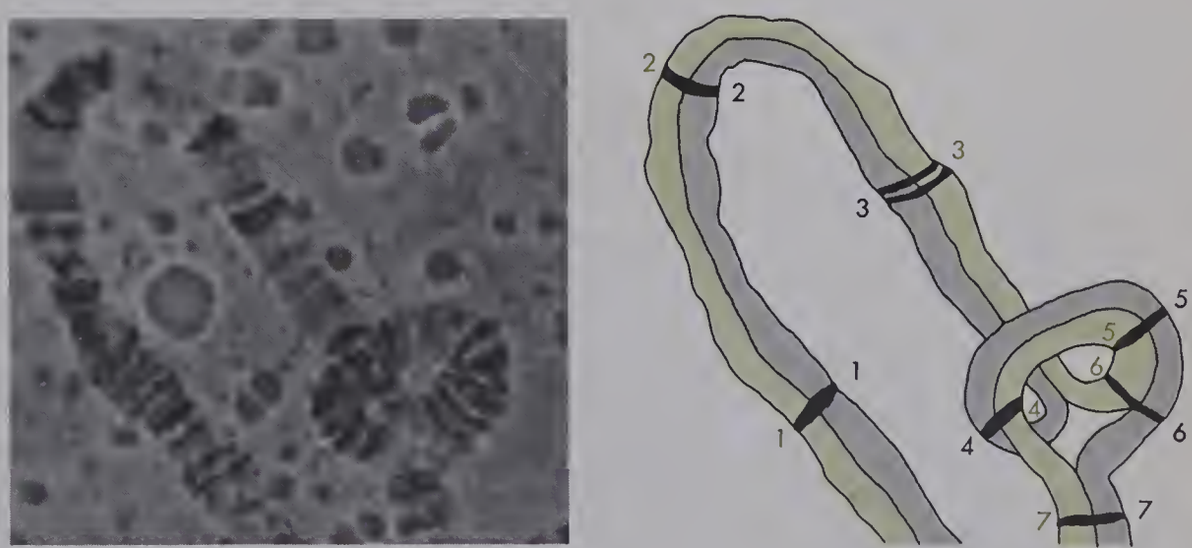


Fig. 31.14 Left: an inversion in one of the No. 3 chromosomes of *Drosophila melanogaster* has caused this loop to form. This inversion was induced by X irradiation, but similar inversions occur under natural conditions. Right: the chromosome carrying the inverted segment ("6, 5, 4") is shown in color; its normal homologue in gray. By forming the loop, the equivalent gene loci on each chromosome can pair with each other. (Photomicrograph courtesy of Edwin Vann, Ph.D., M. D.)

Similar examples can be found throughout the plant and animal kingdoms. Among the most interesting of these are drawn from insects having giant chromosomes. We saw in Chapter 18 with what precision the location of individual genes can be mapped on these chromosomes. We have also seen with what precision equivalent portions of homologous chromosomes pair with each other. Deletions, duplications, and inversions are quickly revealed by the loops they create in the paired chromosomes (Figs. 18.11 and 31.14).

Chromosome pairing in interspecific hybrids

Often (but not always) representatives of two different species of fruit fly can be induced, in the laboratory, to mate. Viable offspring may result although these are usually infertile (like the hybrid mule produced by mating a horse and a donkey). When one examines the giant chromosomes of these *interspecific* hybrids, one finds the explanation of their infertility. Large numbers of mismatched chromosomal regions are present, revealed as chromosome loops (Fig. 31.15). This makes successful meiosis virtually impossible. The remarkable thing from the point of view of our story, though, is the amount of pairing that does take place. Knowing the precision with which this process occurs, we can only conclude that these *different* species share large blocks of *identical* genetic material. (Simple visual inspection of the *pattern* of bands in the chromosomes of the two

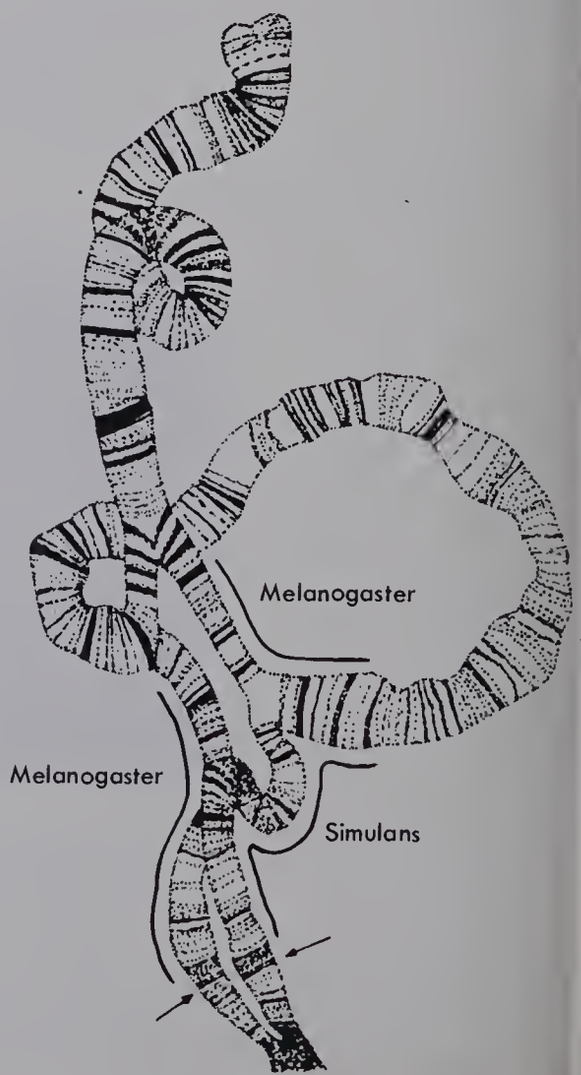


Fig. 31.15 Pairing of the No. 3 chromosomes present in the hybrid produced by mating *Drosophila melanogaster* with *Drosophila simulans*. The chromosomes pair perfectly except where an inversion is present and where there are slight differences in banding pattern (e.g. at the arrows). The extensive identity of these two giant chromosomes indicates that the two species inherited large segments of this chromosome from a common ancestor. (Based on the work of Patau, courtesy of Springer-Verlag.)

species tends to confirm this.) How can we explain this except by assuming that the two species have evolved from a common ancestor from which they each inherited not only individual genes but large blocks of precisely ordered sequences of genes? In fact, detailed analysis of chromosome pairing patterns within and between various *Drosophila* species has revealed how each race and species can be related to the others through a defined sequence of chromosome break-repair events (leading to inversions and translocations). You are urged to read about these analyses in the book by Bruce Wallace cited at the end of the chapter.

31.6 THE EVIDENCE FROM PROTECTIVE RESEMBLANCE

The peppered moth provides evidence of contemporary adaptive change in a species, i.e. evolution

Probably no more dramatic evidence of evolution exists than the spread of **industrial melanism.** Approximately 10% of the more than 700 species of larger moths that are found in the British Isles have been undergoing a dramatic darkening in coloration in regions of heavy industrial activity. This change is called industrial melanism. Perhaps the best-studied example is the peppered moth, *Biston betularia.* The wings and body of this moth used to be light in color with scattered dark markings (giving rise to its common name). In 1848 a coal-black mutant form of the moth was discovered near Manchester, England. Since that time, the black form *(Biston betularia* var. *carbonaria)* has gradually become more prevalent. Today over 90% of the moths in this region are black. Here certainly is an example of a change in a species taking place in nature and rapidly enough to be recorded by observant naturalists.

What accounts for this evolutionary change? *Biston betularia* flies at night and rests by day on tree trunks in the forest. In areas far from industrial activity, the trunks of forest trees are encrusted with lichen growth. As Fig. 31.16 shows so clearly, the light-colored form of the moth is practically invisible against such a background. In areas where air pollution is severe, the combination of toxic gases and soot has killed the lichen growth and darkened branches and trunks. Against such a background, the light-colored moth stands out sharply. *Biston betularia* is preyed upon by birds which pluck it from its resting place by day. In polluted woods, it is easy to see that the dark form would have a much better chance of surviving undetected. In fact, when the English geneticist H. B. D. Kettlewell released marked moths of both kinds in these woods, he was able to recapture twice as many of the dark forms as of the light. Careful observation showed that predatory birds did, indeed, eat a much higher proportion of the light moths released than of the dark. It is no wonder that the dark form is now dominant in these regions. (Just recently, however, follow-up studies have revealed that the light form is *beginning* to make a comeback in the Manchester and London areas. This is probably the consequence of the rigid smoke abatement programs that have been placed in effect in those cities.) In unpolluted areas (e.g. Scotland and southwestern England), the woods are still lichen-encrusted and the light form of *Biston betularia* is still dominant.



Fig. 31.16 Left: the peppered moth *Biston betularia* and the dark form *carbonaria* on a lichen-encrusted tree trunk. Right: both forms of the moth are shown resting on a blackened tree trunk typical of those near industrial areas. (Courtesy of Dr. H. B. D. Kettlewell.)

Other examples of protective resemblance

Industrial melanism is only one example of the development of protective resemblance. There are butterflies which, at rest, look like dead leaves. There are insects which look for all the world like twigs (Fig. 31.17). The robber fly mimics the bumblebee and presumably thereby gains protection from predators. (Note that the robber fly, despite its resemblance to the bumblebee, has not been able to dispense with its evolutionary heritage. Like all flies, it has two wings instead of the four possessed by the bumblebees and their relatives.) We have no reason to believe that these few additional examples of protective resemblance came about any differently from industrial melanism. Evolution explains their existence, too, but in the case of industrial melanism, we have actually been able to see the process of evolution taking place.

31.7 THE EVIDENCE FROM GEOGRAPHICAL DISTRIBUTION

Wallace's six faunal regions

In 1876 the British naturalist Alfred Wallace (who conceived the idea of evolution and natural selection independently of Darwin), proposed that the continental areas of the world could be divided into six main regions on the basis of their animal populations (Fig. 31.18). The greatest diversity of living things is found in the two great tropical areas, the Ethiopian

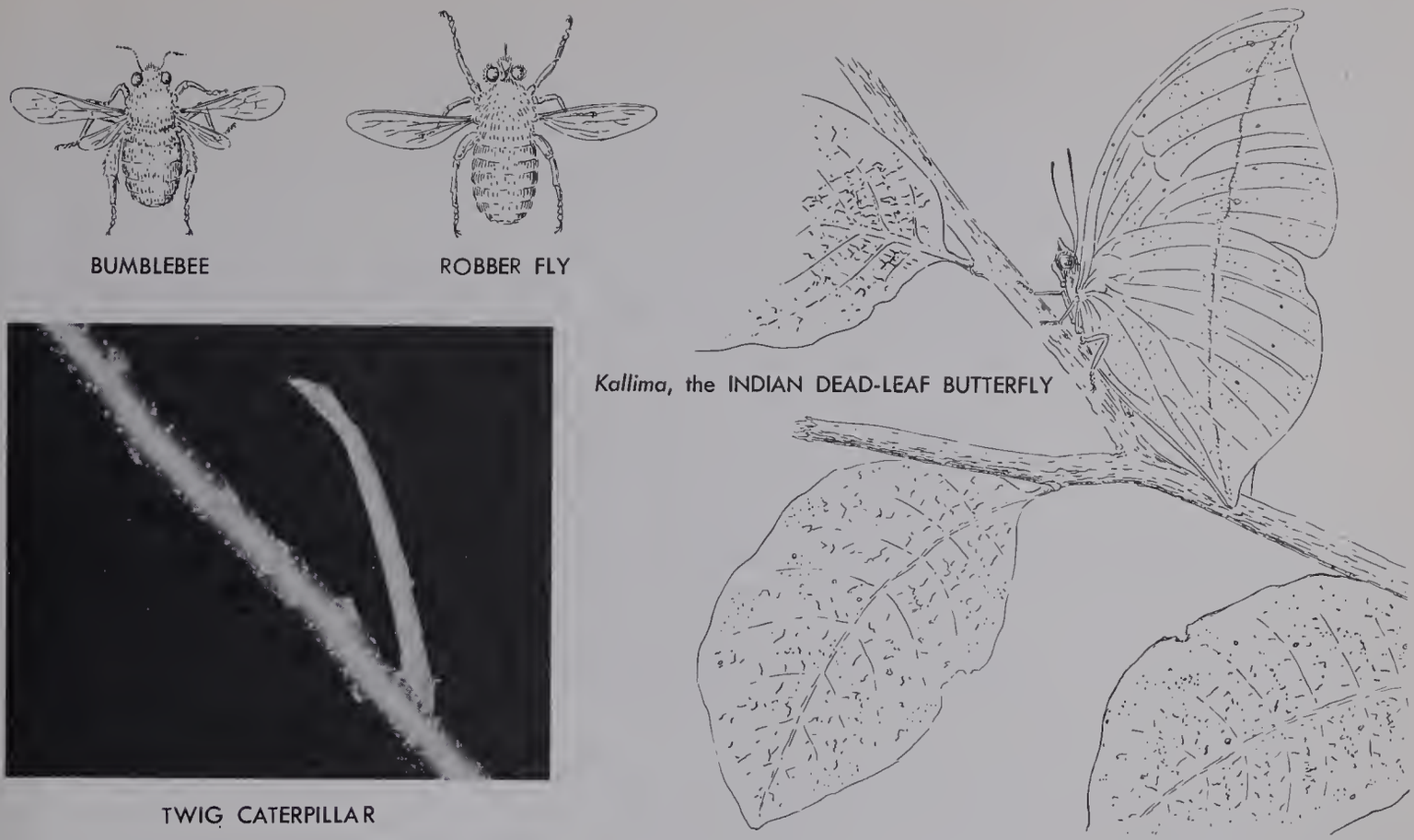


Fig. 31.17 Protective resemblance in insects. The stingless robber fly is more closely related to the mosquito than to the bumblebee. Although the true veins of a butterfly's wing radiate from the point of attachment, *Kallima's* resemblance to a leaf extends even to having a dark line crossing its veins that matches the midvein of a leaf. The motionless twig caterpillar, complete with "buds" and "lenticels," often escapes detection by birds but may occasionally have some other insect lay eggs on it by mistake. (Courtesy of Muriel V. Williams.)



Fig. 31.18 Wallace's faunal regions. The circle on the equator shows the location of the Galapagos Islands.

(tropical Africa) and the Oriental (tropical Asia and the nearby offshore islands). Fossil evidence suggests that in these regions have evolved most of our dominant plants and vertebrates. Europe and northern Asia make up the Palearctic region, while North America is the Nearctic. Spread of plants and animals into and through these regions has often been seriously limited by the harshness of the climate. The two remaining continental regions are the Neotropical (South America) and the Australian (Australia, New Zealand and New Guinea). The unusual animal life (fauna) and plant life (flora) of these regions can be explained by their intermittent isolation from the nearby land masses. The Australian region is isolated right now, of course, while the Neotropical region has been enjoying a land bridge with the Nearctic for the last few million years.

The distribution of plants and animals in the oceanic islands provides particularly strong support for the theory of evolution. Oceanic islands (e.g. the Hawaiian Islands) are those that have never been connected to one of Wallace's continental regions. Many of them have arisen from the sea in relatively recent times (geologically speaking). Nevertheless, they all support a rich and varied fauna and flora. If species were immutable, we should expect that all the creatures to have colonized such islands would be members of species represented on the mainland. As a young man of 26, Darwin visited a group of such islands, the Galapagos Islands off the coast of Ecuador (Fig. 31.18). While the marine birds were those found elsewhere in the Pacific, Darwin found scattered over the islands thirteen species of land birds which were not known anywhere else in the world. At first glance, some of these birds seemed quite diverse (see Fig. 32.5). Some had stout beaks for eating seeds. Others had beaks adapted for eating insects of one size or another. One species had a beak like a woodpecker's and actually used it for drilling holes in wood. However, it lacked the long tongue which our woodpeckers use for removing insects from the wood. Instead, it used a cactus spine, held in its beak, to dig the insect out. Underlying this superficial variety of types was a basic similarity: all these birds were finches. Although one looked far more like a warbler than a finch, its internal anatomy showed its true kinship. Here, then, were a group of birds found nowhere else on earth and all showing definite similarities. This indeed would be a curious exercise in special creation. Far more plausible is Darwin's thought that each of these birds is the result of descent with modification (evolution) from a common finch ancestor which accidentally reached the islands from its original home in South America. Probably nothing else so strengthened Darwin's eventual conviction that species are changeable and are the product of evolution than the example of his finches.

The Galapagos finches

Even in cases in which a species is uniformly distributed through a large region, we find evidence of evolution at work. The common leopard frog (*Rana pipiens*) is found throughout the eastern portion of North America. At no place in its range can one sharply divide this population into northern forms and southern forms. Nonetheless, significant differences in physiology and behavior exist between northern and southern animals. In

The existence of clines contradicts the idea that species are immutable

fact, if one breeds Vermont frogs with Texas ones, the offspring develop abnormally. This certainly suggests that genetic differences exist between the specimens so great as to prevent successful hybrid formation. With a Vermont frog in one hand and a Texas frog in the other, one would be quite justified in assigning them to separate species. But at every place from Vermont to Texas leopard frogs are breeding freely with one another and clearly constitute a single species. Such a continuous distribution of animals or plants which vary gradually from one end of their range to the other constitutes a **cline.** It provides irrefutable evidence that species are not necessarily uniform and unchangeable groups of organisms, hence lending strong support to the theory of evolution.

31.8 THE EVIDENCE FROM DOMESTICATION

Man has deliberately manipulated the evolution of many animal and plant species

The deliberate cultivation of plants and animals has occupied man for thousands of years. During all of this time, but especially in the last two centuries, he has developed varieties or breeds of plants and animals which yield more and better food or which, in other ways, better serve his purposes. While, with rare exceptions, he has not created new species in this process of domestication he has certainly created forms which differ greatly from the ancestral stock. The extraordinary diversity of the domestic dog, from Chihuahuas to Saint Bernards, provides dramatic evidence of man's ability to alter a species by selective breeding procedures. In fact, the corn plant (*Zea mays*) has been altered so greatly in the course of domestication that it can no longer survive without the aid of man. The many breeds of horses, cows, goats, sheep, chickens, and rabbits, which can be seen at any country fair, are dramatic testimony to the variability of species and man's ability to create evolutionary changes for his own benefit.

The standard by which a theory is judged is its ability to explain the *most* facts in the simplest manner and to make possible the prediction of new facts. The slowness with which most evolutionary change occurs has hampered the theory of evolution in the second respect. In its ability to provide a simple, comprehensive explanation for a vast array of facts, however, the theory of evolution justly stands as the most important theory in biology. Every aspect of the living world that man studies, from biochemistry and cytology to anthropology and history, has been nourished and broadened by it.

While the geographical distribution of animals may have provided Darwin with his most compelling evidence that evolution has occurred, it was his study of the process of domestication that gave him the clue as to how it occurs. It is his theory of the mechanism of evolutionary change which truly makes his *Origin of Species* such an epoch-making work. We shall turn our attention to this theory in the next chapter.

EXERCISES AND PROBLEMS

1. What has happened to most of the fossils that were formed during the history of life?
2. The theory of recapitulation is often stated: "ontogeny recapitulates phylogeny." Check the meaning of these three words in the glossary.
3. Does the statement of the theory of recapitulation in Question 2 accurately describe the facts? Explain.
4. Which two of the six animals whose embryonic stages are illustrated in Fig. 31.10 do you think have most recently shared a common ancestor? Explain.
5. How would you go about preparing antibovine (anticow) antibodies?
6. Frogs (*Eleutherodactylus*) were not found in Bermuda before being introduced by man in 1880 but were prevalent in the islands of the West Indies. Can you explain why they were found in one location and not the other?
7. Would you predict on the basis of the data in Fig. 31.13 that we are more closely related to the Old World monkeys or the New World monkeys? Explain.
8. The evolutionary history of mollusks and reptiles is known much better than that of flatworms. Why?

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32 EVOLUTION: THE MECHANISM

Inheritable Variation: the Raw Material of Evolution

Natural Selection

The Measure of "Fitness"

The Genetic Source of Variability

The Hardy-Weinberg Law

When the Hardy-Weinberg Law Fails to Apply

The Effects of Increased Selection Pressure

The Effects of Relaxed Selection Pressure

What is a Species?

The Role of Isolation

Reunion

Speciation by Polyploidy

Convergent Evolution

32.1 INHERITABLE VARIATION: THE RAW MATERIAL OF EVOLUTION

The techniques of domestication suggested to Darwin the mechanism of evolution

Twenty-two years elapsed from the time that Darwin returned home convinced of the truth of evolution and the actual publication of the *Origin of Species.* During this long interval, Darwin accumulated still more data to bolster his case. Living in rural England, he had plenty of opportunity to watch local farmers practice the art of animal breeding. Art it was, because the science of genetics was still unborn. Nonetheless, improved varieties of all sorts of domestic animals were being created. Darwin, recognizing these as evolutionary changes, sought the mechanism by which they were produced. He found that animal breeders could only develop new breeds from variants that appeared naturally in their livestock. Only if some animals were born heavier or taller or darker-coated than others could the breeders develop heavier or taller or darker-coated breeds. Darwin also realized that these variants could not arise simply from exposure to a different environment. Individuals that are heavier simply as a result of better diet could never supply the raw materials for a heavier breed. Only **inheritable variations** could be used for this purpose.

The second vital ingredient in the formation of new varieties was **selective breeding.** Once individuals with desirable inherited traits were identified, they were mated together in hopes that (1) a higher proportion of their offspring would have the trait than in the population as a whole and (2) the expression of the trait would be intensified in the offspring. By repeating this process of selective breeding, it eventually was possible to develop pure-breeding lines displaying the new trait. The various breeds of horses, cattle, swine, dogs, and cats, to mention just a few, have been produced in this manner.

Man, then, can bring about evolutionary change. Does a similar mechanism exist in nature? The first thing we must look for in populations of wild organisms is inherited variations which can serve as the raw material for evolution. Anyone who has studied a large sample of individuals in a single species knows that variation does exist in nature. Such variation takes two forms.

1. Continuous Variation. Many traits found in a population of plants or animals vary in a smooth, continuous way from one extreme to the other. Body weight, body length, and coat color are just three traits in which we might expect to find a whole range of variations. Typically, most of the individuals fall near the middle of the range with fewer individuals at the extremes (Fig. 32.1). You can demonstrate the existence of this type of variation right around you. Although your classmates may not qualify as a wild population, calculation of their heights or weights (of one sex) will reveal a wide, unbroken range of values with more individuals in the middle of the range than at the extremes. These data can be plotted on a graph (look back at Fig. 17.9) and the curve that results is typical of those obtained by studying continuous variation in wild populations.

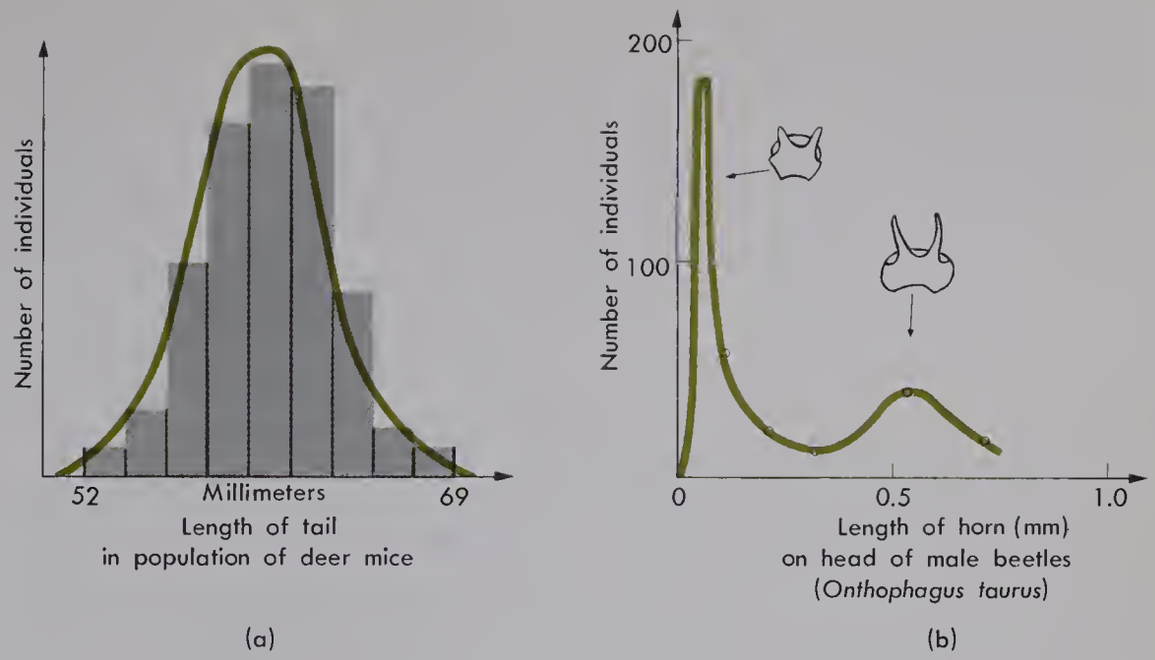


Fig. 32.1 Examples of (a) continuous variation and (b) discontinuous variation. (Redrawn by permission from D'Arcy W. Thompson, *On Growth and Form,* Vol. 1, Cambridge University Press, 1959.)

Polymorphism

2. Discontinuous Variation. With respect to certain traits, the individuals of some populations fall into two or more distinct categories with no intergrading between them. The various castes in a honeybee colony (see Section 29.8) are an example of such discontinuous variation. The fact that all humans have one of four possible ABO blood types is another. Such variation is called **polymorphism.** The light and dark forms of *Biston betularia* (Fig. 31.16) illustrate polymorphism, too. There are no gray individuals. Plotting the distribution of polymorphic traits, one gets not a single bell-shaped curve but discrete peaks for each form of the trait (Fig. 32.1).

Both continuous and discontinuous variation can provide the raw materials for evolution, but only if they arise from hereditary rather than just environmental factors. Perhaps the continuous variation in the height of your classmates is simply a reflection of variation in their diet as infants. We already have seen that the development of supplementary egg-laying females in a honeybee colony deprived of its queen is controlled by the withdrawal of a chemical substance. How, then, can we determine whether variation in a wild population is to some degree inherited? As a practical matter, this is often very difficult. It involves the mating of individuals both of whom are extreme examples of the trait under study. If, for example, the offspring of two extra-large mice are significantly larger than the average for the population, hereditary factors are undoubtedly at work (Fig. 32.2). On the other hand, if the offspring occupy the same range as the average for the population, environmental factors are working alone. A population of bean seeds of a pure strain varies over a range of several mm in length. If extra-small beans are crossed, however, the new crop will show no shift toward a smaller size. Variation in size, then, does not seem to be inherited in this particular case.

Estimating to what degree observed variation is under genetic control

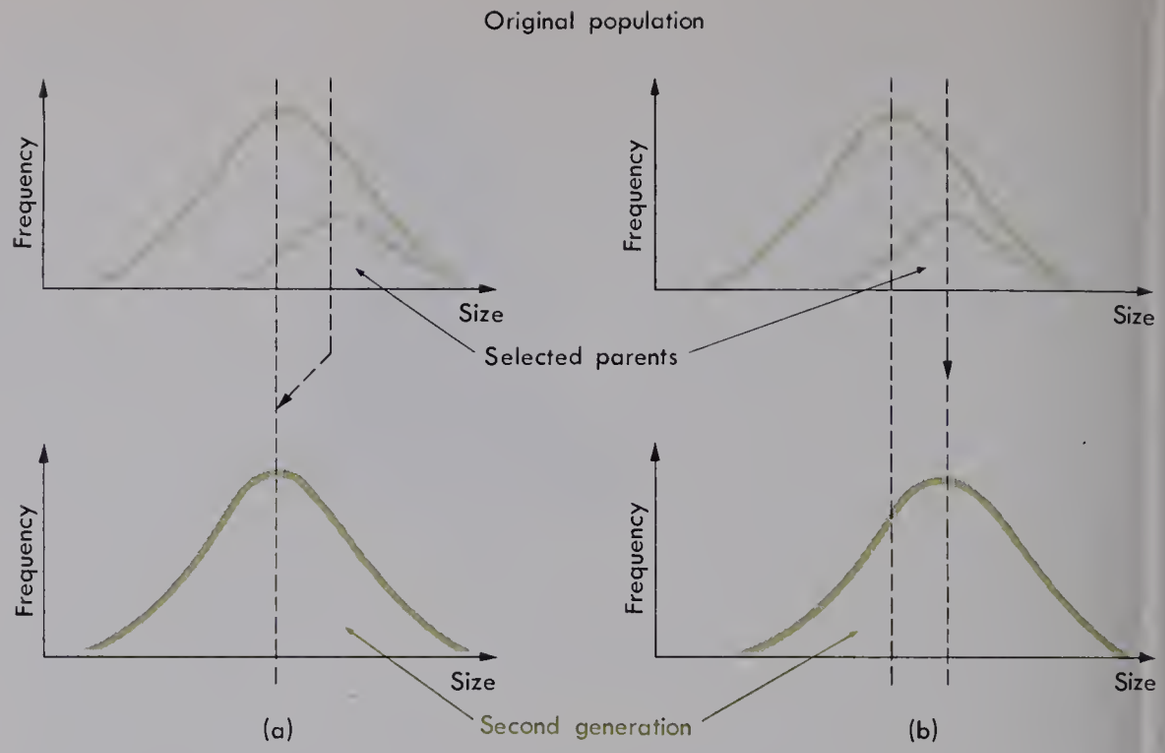


Fig. 32.2 Effect of selective breeding when variations are caused by (a) the environment and (b) heredity.

32.2 NATURAL SELECTION

Granted that inherited variability does occur in wild populations, is there a mechanism in nature to accomplish the selective breeding that Darwin saw was an indispensable part of the process of domestication? The answer to this question and the key to the whole theory of evolution came to Darwin as a result of reading the *Essay on Population* by the English clergyman Thomas Malthus. In this work, Malthus pointed out the remarkable reproductory potential of all organisms. We have already seen that the American oyster may release a million eggs in a season and a single puffball fungus may release 700,000,000,000 spores in the same period. Obviously, the earth would quickly be overrun with oysters, puffballs, in fact even the slowest-breeding plants and animals, if their populations were not kept in check by some means. Darwin called this means **natural selection.**

His theory of natural selection is a conclusion based upon three observable facts of nature and one preliminary conclusion. We can summarize the theory as follows:

Fact No. 1. All species have a high reproductory potential. From oysters to elephants, they are capable of filling the earth with their kind.

Fact No. 2. Except for minor fluctuations, the population of any given species remains fairly constant from year to year.

Conclusion No. 1. Therefore, we must conclude that all creatures face a *continual struggle for existence.*

Fact No. 3. There is inherited variation among the individuals of any species.

Survival of the fittest

Conclusion No. 2. Therefore, we may conclude that those individuals whose variations best fit them for their environment will be most likely to survive. This idea of the *survival of the fittest* is what we call Darwin's theory of natural selection. It is the mechanism Darwin thought accomplishes in nature what man's selective breeding accomplishes in domestication.

32.3 THE MEASURE OF "FITNESS"

Fitness is measured by one's success at leaving descendants

Darwin's theory of natural selection has often been seriously misunderstood. To many people, it has implied that a "dog-eat-dog" existence is natural and hence justifies man's most ruthless exploitation of his fellow man and of the other resources of his environment. Actually, fitness measured in terms of aggressiveness applies to only a portion of the world of living things and even then is only one of many criteria involved. Fitness as a biological concept can be measured only in terms of the ability to produce mature offspring. The fittest organisms in any population are those that leave the largest number of mature offspring. A variety of mechanisms may play a role in this.

1. Survival. The most important is simply the ability to stay alive until one's reproductory age is over. (Many organisms, as we have seen, continue to reproduce as long as they live. These include the perennial plants, many invertebrates, fishes, amphibians, and reptiles.) Any trait which increases the organism's chances of surviving to and through this period makes that organism more "fit" than the others of its species. We call such traits **adaptations.** They may involve a change in body structure, a change in body physiology, a change in behavior, or all of these. While strength, long claws, fangs, etc., do promote survival in some cases, this kind of adaptation probably plays a minor role in the evolution of living things. For every adaptation associated with aggressive behavior, one can cite literally dozens of examples of adaptations which play a more inconspicuous, but every bit as important, role in promoting survival. The mimicry of the Indian dead-leaf butterfly, the twig caterpillar, and the robber fly (Fig. 31.17) are examples of morphological adaptations that make their owners more fit by reducing the likelihood of their being eaten by predators. The melanism of *B. betularia* var. *carbonaria* similarly promotes survival and hence makes the form more "fit." In fact, any change which merely improves the efficiency of an organ promotes survival. In a real sense, this entire book has been a catalogue of adaptations that confer fitness on the individuals having them.

Adaptations

Physiological and behavioral adaptations go hand in hand with morphological ones. The ability of reptiles to excrete their nitrogenous wastes as uric acid is dependent upon the development of a nephron in which glomerular filtration is reduced and tubular excretion increased (see Section 15.11). Similarly, the mimicry of the Indian dead-leaf butterfly would be of no avail if this creature did not make a practice of resting quietly on

twigs with its wings closed. The remarkable morphology of the twig caterpillar would have no survival value if this creature spent its time moving about on the broad surfaces of leaves.

The fact that natural selection can operate only on traits that promote survival through the reproductory years has interesting consequences for those organisms, such as man, in whom reproduction usually ceases long before death. Any traits that appear for the first time after reproduction has ceased cannot be selected for, because no mechanism remains for their gradual increase in the population. Of course, traits that improve well-being late in life may improve fitness early in life, too. However, the absence of any specific selection of traits that improve physical and mental health late in life may well account for the sharp rise in degenerative changes that occur in old age. It may also account for the fact that despite the dramatic increase in the average life span of Americans and Europeans during the last half century, our *maximum* life span remains virtually unchanged.

2. Sexual Selection. Another factor affecting "fitness" is sexual selection. Any inherited trait that makes some individuals more desirable to the opposite sex than others will thus make them more successful in mating. Consequently, a larger proportion of the next generation will have inherited this trait. Darwin was familiar with many examples of sexual selection. Brilliant plumage in male birds is a trait which may often be explained on this basis.

An experimental demonstration of sexual selection

Sexual selection probably operates in a negative as well as a positive way. Certain structural variations may be less successful in "releasing" mating behavior in individuals of the opposite sex. In 1950, the husband-and-wife team of Sheldon and Elizabeth Reed set up a culture of fruit flies containing equal numbers of red-eyed males, red-eyed females, white-eyed males, and white-eyed females. Although previous experimentation had shown that white-eyed flies are just as healthy and live just as long as red-eyed flies, after 25 generations there was not a white-eyed fly to be found in the culture. These investigators had noted that not only did red-eyed females prefer red-eyed males but white-eyed females did, too. Such sexual selection was strong enough to eliminate the less "fit" variants in a remarkably short span of time.

Inverse relationship between number of young and care given to them

3. Family Size. Any trait resulting in the production of larger mature families is also a measure of fitness. It is necessary to stress mature families because only mature offspring will be able to pass these traits on to still another generation. In animals, such as fish and oysters, which provide little or no care for their young, the way to larger mature families is through a larger number of newborn. In these species, fitness is measured by the number of fertilized eggs they can produce. On the other hand, in species where the young are cared for by their parents, selection acts to reduce family size. Assuredly, if humans had as many offspring as oysters, they could not begin to care for their young, and fewer, rather than more, adults would be produced. With respect to family size, then, the "fittest"

individuals are those with the largest families at the end, not necessarily at the beginning.

One of the criticisms leveled at Darwin's theory of natural selection was that it failed to provide any explanation of how desirable traits are passed on from generation to generation. In other words, Darwin was not able to explain the mechanism of inheritance. Such a criticism was hardly fair. As you know, the science of inheritance (genetics) had to await the rediscovery of Mendel's work at the beginning of this century. Furthermore, so long as it is granted that natural selection can work only on inheritable variation, our not knowing what the mechanism of inheritance is does not weaken the theory of evolution. In any case, today we do understand many of the principles of genetics, and they have indeed greatly increased our appreciation and understanding of the mechanism of evolution. Evolution involves a gradual change in the traits of a species. But traits are the expression of the genotype, and thus evolution must also involve a change in the genotypes of the individuals in the species. Let us now analyze the theory of natural selection in the language of genetics.

32.4 THE GENETIC SOURCE OF VARIABILITY

How genes are reshuffled

Genetics gives us the means to understand the origin of inherited variability in populations. The process of **sexual reproduction** creates new gene combinations, new genotypes, and hence new phenotypes or variants in the population. These new gene combinations originate in three ways: (1) At the time of gamete formation (spore formation in plants), **crossing over** of homologous chromosomes during meiosis produces new combinations of genes. (2) At metaphase of meiosis, **random assortment** of the homologous chromosomes on either side of the equatorial plate provides a further reshuffling of the genes inherited from the individual's parents. (3) The union of gametes from genetically dissimilar individuals, **outbreeding,** is also a source of variability as the maternal and paternal genes interact to produce a new phenotype, a new, unique individual.

Mutation is the source of new genes

Underlying these three processes is **mutation.** If all the individuals in a population were homozygous for the same genes at every gene locus, no amount of genetic "reshuffling" would create anything new. It is mutation that produces new alleles which can then be combined in various ways with existing genotypes.

Most mutations that geneticists have studied are harmful. This has led many people to question how the process of mutation can provide for progressive evolutionary change. The solution to this puzzle is probably historical. Species alive on the earth today are the products of three billion or more years of evolutionary change. With natural selection operating over such a vast span of time, it should not be surprising that the genes still present have proved to be of definite survival value. Any change in a gene now is far more likely to produce a harmful effect than a helpful one.

The evolutionary significance of diploidy and haploidy

In comparing the animals and higher plants with the lower plants (e.g. mosses) and most microorganisms, we find that the diploid generation is dominant in the former while many of the latter are haploid during the major part of their lives. If we can assume that the higher plants and animals represent a more elaborate and more successful sequence of evolutionary changes, the occurrence of diploidy may well be a factor in this. Recessive genes, as you have learned, often remain "hidden" in heterozygous individuals, the dominant allele having full expression in the phenotype. This is, however, true of diploid organisms only. In haploid organisms there is only one gene present for each gene locus. Consequently, recessive genes express themselves in the phenotype. Natural selection operates only on genes which affect the phenotype. Hence, we can expect natural selection to work more vigorously on the genes of haploid organisms than of diploid. Hidden in the recessive condition, a moderately disadvantageous gene in a diploid organism gets a chance to spread in the population and to be reshuffled in various combinations with other genes. Thus it gets a chance to prove itself advantageous in some combinations or in some environments instead of being quickly weeded out of the population by natural selection.

Our study of genetics also provides us with an understanding of the basis of hereditarily controlled variation, both continuous and discontinuous. Continuous variation that is under hereditary control arises from **multiple factors** (see Section 17.9). The distribution of multiple factors in the offspring of heterozygous parents forms a bell-shaped curve similar to the one we found that describes continuous variation as it occurs in nature. Discontinuous variation (polymorphism), on the other hand, probably is controlled by a single gene locus (e.g. human ABO blood types) or, in the case of differences associated with sex (e.g. bright plumage in male birds), the presence or absence of a sex chromosome.

Population genetics

In applying the discoveries of genetics to the subject of evolution, we must consider the genotypes of entire populations rather than just individuals. This is known as **population genetics.** Students often experience some difficulty in applying the genetics of individuals to populations. For example, one common error is to assume that any phenotype (such as blood Type B) which is present in only a small portion of the population will ultimately disappear. Many students seem to feel intuitively that a gene, such as L^B, which is present in only a small percentage of the population will eventually be swamped by its more prevalent allele. But intuition is insufficient in this case. Let us see why.

32.5 THE HARDY-WEINBERG LAW

In mating two individuals heterozygous (hybrid) for a given trait, we found that the F_2-generation consists of 25% homozygous dominants, 50% hybrids, and 25% homozygous recessives. We determined this by setting up a Punnett's square (Fig. 32.3). On the left-hand side and across

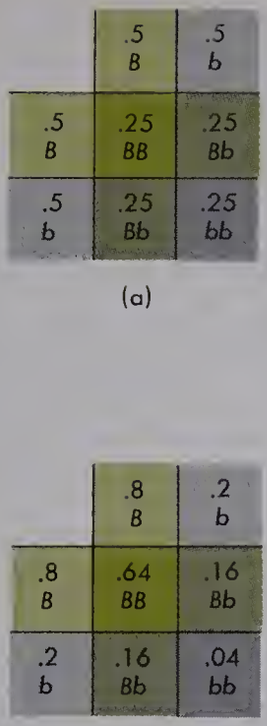


Fig. 32.3 (a) Results of random union of the gametes produced by two hybrids. (b) Results of random union of the gametes produced by an entire population with a gene pool containing 80% *B* and 20% *b*.

the top, we placed symbols representing the genotypes of the two kinds of gametes that can be formed by these individuals. Implicit in this was the fact that the two kinds of gametes were present in equal numbers, i.e. 50% of the sperm (or eggs) contained the dominant allele, 50% the recessive. But in an entire population of breeding organisms (homozygous dominants, homozygous recessives, as well as hybrids), the total number of gametes that contribute to the formation of the next generation will in most cases not be equally divided between those carrying the dominant allele and those carrying the recessive one. As a hypothetical case, let us examine a population of hamsters in which 80% of all the gametes formed carry the dominant allele for black coat color (*B*) and 20% carry the recessive allele for gray coat (*b*). What genotypes will be produced in the next generation? We can set up a Punnett's square for this problem, too, but in this case we must be explicit about the percent of each allele in the "pool" of gametes. Eighty percent of the sperm carry *B* and fertilize eggs at random. Eighty percent of these also carry *B*, so we predict that 64% (.80 X .80 = .64) of all the zygotes will be *BB*. Twenty percent of the sperm carry *b* and fertilize the eggs at random. Eighty percent of these, you remember, carry *B* and so we would expect 16% of the zygotes formed to result from this combination and to be heterozygous (*Bb*) for the trait. Another 16% of the zygotes would also be heterozygous as they would result from the 80% of *B* sperm fertilizing the 20% of *b* eggs. Only 4% of all zygotes produced would be homozygous for gray coat (*bb*) since the 20% of the sperm carrying allele *b* had only a 1-in-5 chance of fertilizing an egg carrying *b* (.20 X .20 = .04).

The result, then, of random mating in such a population of hamsters would be a generation of offspring 96% of whom had black coats and only 4% gray coats. Here is a situation where intuition might suggest that gray-coated hamsters will gradually disappear. Let us see what happens, though, when this generation is mated. Sixty-four percent of all the hamsters, both male and female, will contribute only *B*-containing gametes as they are homozygous for *B*. Half of the gametes produced by the 32% heterozygous individuals, in other words 16% of the total, will also contain the *B*-allele. None of the gray hamsters will contribute *B*-containing gametes, of course. Therefore, of the entire pool of gametes available for creating the next generation, 80% will contain the *B*-allele (Fig. 32.3).

All of the gray hamsters (4% of the population) will contribute the *b*-allele to the pool of gametes. In addition, half of the gametes produced by the heterozygous individuals also contain the *b*-allele. Consequently, a total of 20% (4% + 16%) of all the gametes will contain this allele.

Bringing these gametes together at random, we find that we have duplicated our first situation exactly. Once again, a generation of 96% black hamsters and 4% gray hamsters will be formed. The proportion of allele *b* in the population has not diminished but has remained at the same level. The heterozygous individuals, although of black phenotype, help ensure that each generation will contain four gray hamsters out of a hundred.

The gene pool

Our modified Punnett's square is a little cumbersome for dealing with population genetics. The same results can be achieved by using the mathematical relationship $(p + q)^2 = p^2 + 2pq + q^2$. (Perhaps you remember from your study of algebra that $p^2 + 2pq + q^2$ is called the expansion of the binomial $(p + q)^2$.) The total number of genes in a population is its **gene pool.** If p represents the percent of one gene in the gene pool and q represents the percent of its *single* allele ($p + q$ must always equal 1—why?), the genotypes of the next generation can be quickly computed: p^2 equals the percentage of the population homozygous for the dominant gene; q^2, the percentage homozygous for its recessive allele; and 2 pq the proportion of heterozygous individuals in the population. In our example, $p = .80$, $q = .20$, and thus

$$(.80 + .20)^2 = (.80)^2 + 2(.80)(.20) + (.20)^2 = .64 + .32 + .04.$$

This is precisely the result that we obtained by using our modified Punnett's square.

Not the least advantage of the algebraic method, rather than the Punnett's square method, is that we can work backward as well as forward. In fact, we were really putting the cart before the horse when we selected a population with a gene pool containing 80% *B* and 20% *b*. The only way this value can be established is by determining the percentage of the recessive phenotype and computing from it the value for q. In the case of our hamsters, $q^2 = .04$, $q = \sqrt{.04} = .20 = 20\%$, the percentage of *b*-alleles in the gene pool of the population. Since $p + q = 1$, $p = 1 - q$ and allele *B* must constitute 80% of the gene pool. Because *B* is completely dominant over *b*, we cannot distinguish the hybrids from the homozygous dominants by their phenotype. However, substituting in the middle term ($2pq$) of our expansion, we can determine the percentage of hybrids in the population:

$$2pq = (2)(.80)(.20) = .32 = 32\%.$$

In cases of *incomplete dominance* in such a polymorphic species, the three genotypes will, of course, each give a distinct phenotype.

The results of our calculations show that recessive genes do not tend to be lost from a population no matter how small their representation in the gene pool. Stated more generally, we can say that so long as certain conditions (to be discussed below) are met, gene frequencies in a sexually breeding population remain constant from generation to generation. This is known as the *Hardy-Weinberg law* in honor of the two men who first realized the significance of the binomial expansion to population genetics and hence to evolution.

The maintenance of constant gene frequencies provides a reservoir of variability in the population

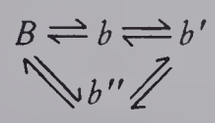
What are the implications of the Hardy-Weinberg law for evolution? Certainly the mechanism described by the law is not an evolutionary one. Evolution involves changes in the gene pool, and this is precisely what does not occur with the conditions under which the Hardy-Weinberg law operates. What the mechanism does provide is a **reservoir of variability** so that if future conditions require it, the genetic constitution of the popu-

lation can change. If recessive alleles were continually tending to disappear from a population, the population would soon become homozygous. As we have seen, such a uniform genotype would provide no variability and hence no opportunity for evolutionary change. On the other hand, under the conditions of the Hardy-Weinberg law, genes which may be of no particular value to a population will nonetheless be retained. At some future time, these genes might hold the key to survival in a changed environment, and their presence then could enable the population to avoid extinction.

32.6 WHEN THE HARDY-WEINBERG LAW FAILS TO APPLY

To see what forces lead to evolutionary change, we must examine the circumstances in which the Hardy-Weinberg law may fail to apply. There are four:

1. Mutation. In order for the Hardy-Weinberg law to apply, either there must be no mutations occurring in the population or the rate of forward and back mutation must be in equilibrium. The latter qualification is the only one that really interests us because mutations are continually occurring in all populations. Genes vary in their mutability but, on the average, a given gene locus undergoes a "spontaneous" mutation once in every 100,000 to 1,000,000 duplications. The number of mutations can be increased by appropriate mutagenic agents (see Section 19.3) but, even so, the mutation of any given gene locus is strictly governed by chance. We can increase the likelihood of a given gene's mutating by increasing the over-all mutation rate, but we cannot force a selected gene locus to mutate. A gene which mutates from, say, B to b may at a later time back-mutate from b to B. Just so long as one back-mutation occurs for every forward mutation, the processes are in equilibrium and the Hardy-Weinberg law still applies. Note that this is not the same as saying that at equilibrium the gene pool will contain 50% B and 50% b alleles. A banana placed in a box with 100 flies may soon have 89 flies walking on its surface while the remaining 11 fly about. If every time a fly leaves the banana, another takes its place, the forward and back processes are in equilibrium. Note, too, that the presence of multiple alleles (see Section 19.8) suggests that mutation may occur in more than one direction. But no matter how many alleles



are involved, equilibrium can be reached throughout the system and, at such time, the Hardy-Weinberg relationship will again hold true. Mutation, then, by itself seldom causes the gene pool to shift in one direction. Mutation is not evolution but it does provide the raw materials of evolution. A variety of alleles is made available in the gene pool.

Mutations provide the raw material of evolutionary change

2. Random Genetic Drift. Although many species of plants and animals have large, widespread populations, the number of individuals with which any one plant or animal may mate is usually limited to those in the immediate vicinity. Insect-pollinated angiosperms are apt to be fertilized by individuals growing relatively near to them. Despite their powers of locomotion, animals also tend to breed in a rather restricted area and hence have available a relatively small number of potential mates. Careful field studies show that most small mammals and insects stay close to the area in which they were born. Even birds, which may migrate over thousands of miles, usually return, season after season, to the same small area for breeding.

If geographical barriers also prevent the members of a small local population from breeding with individuals outside the locality, the local population becomes genetically isolated from the rest of its species. The gene pool of a population that combines small size and genetic isolation may not observe the Hardy-Weinberg equilibrium. Let us see why.

Small populations may not observe the Hardy-Weinberg law

In discussing Mendel's experimental results (see Section 17.4), we noted the importance of a large sample in making a sound statistical analysis. A coin flipped ten times may very likely not show five heads and five tails. So it is with small breeding populations. The Hardy-Weinberg law is a statistical law and, in order to be valid, a sample size sufficiently large to minimize chance deviations must be taken. A hundred or fewer breeding pairs may very well not reassort their genes according to our expectations. Chance may eliminate certain individuals (e.g. homozygous recessives) *out of proportion* to their numbers in the population. In such a case, the frequencies of certain genes in the population may begin to drift toward higher or lower values. Ultimately they may represent 100% of the gene pool or, just as likely, disappear from the population entirely. This phenomenon is known as random genetic drift. Its significance to evolution was first made clear by the American geneticist Sewall Wright.

Random genetic drift produces alterations in the gene pool of the population, and hence it produces evolutionary change. However, unlike Darwin's idea of evolution, the new individuals in the population may very well be no more "fit" than their ancestors. Evolution by random genetic drift is aimless, not progressive.

Granted that many breeding populations of plants and animals are small enough for random genetic drift to occur, is there any evidence that it does? This is a difficult question to answer, for who can say whether an observed change in the gene pool came about because it conferred greater "fitness" on the individuals or simply as a result of random genetic drift?

Blood group frequencies in certain populations suggest the occurrence of random genetic drift

Although it may not invariably be true, geneticists generally feel that no combination of L^A, L^B, and l blood-group alleles in the human population confers any significantly greater degree of fitness than any other. Therefore, marked changes in the distribution of these alleles might indicate the operation of genetic drift. Some years ago, a study of genetic traits, including blood type, was made of a religious sect called the Dunkers, many of whose members live in Franklin County, Pennsylvania. These people (for-

mally known as the Old Order German Baptist Brethren) have, since the establishment of their sect in West Germany in the early eighteenth century, tended to marry exclusively within the group. In the communities studied, the number of pairs of parents in each generation has been about ninety, a breeding population small enough for random genetic drift to occur. Sixty percent of the Pennsylvania Dunkers studied had blood type A. In the United States population as a whole, the figure is 42%. In West Germany, the region from which the Dunkers emigrated, the figure is 45%. We can rule "fitness" out as a factor here because the non-Dunkers living in the same region show no such preponderance of A-type blood. Some marriages outside the group have occurred, but these would naturally tend to reduce the percentage of the L^A allele in the population. Despite this tendency, the Dunkers display a distribution of blood types found elsewhere only in such groups as Eskimos, Polynesians, and American Indians. We may confidently conclude then that random genetic drift has accounted for this unusual pattern.

Random genetic drift reduces the diversity of the gene pool

Just how important a role random genetic drift plays in evolution is not yet known. It seems probable that where drift does occur in a small population, variability in that population diminishes. In other words, it is apt to lead to the complete loss of certain alleles from the gene pool. In this respect, then, random genetic drift works counter to the force of mutation. Mutation produces a new supply of alleles, a source of variability, while drift results in the elimination of all but one (*which* one cannot be predicted) at a given gene locus.

Although random genetic drift leads to loss of variability in a particular population, it may increase variability within the species as a whole. Small, isolated populations may develop traits quite different from those generally characteristic of the species. With a sudden environmental change, these new traits might enable the small group to survive while the members of the larger population succumbed.

3. Gene Migration. The total isolation of a local population represents an extreme that probably occurs rarely. On the other hand, there are species with large, wide-ranging populations in which distinctive, local subpopulations do not occur at all. Between these two extremes, we find many cases where local populations within a species do become established. The members of these populations generally breed only with one another, but from time to time they may breed with immigrants from adjacent populations of the same species. Although a given local population may have its own distinctive gene pool, when immigrants arrive from other populations with different gene pools, new genes are introduced. This phenomenon is called gene migration. It acts to maintain genetic variability in small populations that might otherwise lose it by random genetic drift or natural selection.

Gene migration increases the diversity of the gene pool

It is usually difficult to detect evidence of gene migration unless the adjacent subpopulations differ in some easily visible trait controlled by one or a few gene loci. Such a situation occurs with the dark and light forms

The peppered moth shows evidence of gene migration

of the peppered moth, *Biston betularia,* which differ from one another by a single gene. In general, the proportion of dark forms in an area is related to the degree of industrial pollution in that area. However, the moth population in some polluted areas has a higher proportion of light forms than would be expected. Where this situation has been analyzed, geographical barriers (such as an intervening body of water) separating that particular population from adjacent dark populations have been more effective in preventing interbreeding than the barriers separating it from adjacent light populations. Gene migration from the light population has prevented the population in question from showing the proportion of dark forms that natural selection in its habitat would normally be expected to produce. (The light population may also be affected by migration of the gene for melanism into *it.*)

Just how important gene migration has been in the evolution of animals is debatable. In plants, it seems to have played a significant role. Here we find that it occurs not only between subpopulations of the same species but, even more important, between different (but still related) species.

Introgression is gene migration between species

Breeding between species is called **hybridization** and it is quite commonly observed in plants. When the hybrid formed by the mating of individuals of two different species then breeds with one of the parental types, new genes are passed into the gene pool of that parent. This phenomenon is often called introgression, but it is simply a case of gene migration *between* species rather than *within* them.

Many good examples of hybridization and introgression have been studied. It occurs most often in areas where the habitat has been so disturbed (usually by man's gardens, roads, pastures, etc.) that hybrid individuals can survive there as well as or better than either parental type. The many "weed" species, which are so characteristic of unsettled habitats, are particularly apt to show its effects. One species of wild lettuce, a weed common along our roadsides, often shows signs of genes acquired by hybridization with the domesticated species growing in nearby gardens. As a result of repeated crossing, these genes spread through the wild-lettuce population, giving rise to increased variability as they go. Gene migration, then, in contrast to random genetic drift, brings about increased rather than decreased variability in the gene pool.

Natural selection is differential reproduction

4. Differential Reproduction. The fourth circumstance under which the Hardy-Weinberg law fails to apply occurs when differential reproduction takes place. If individuals having certain genes are better able to raise large mature families than those without them, the frequency with which those genes appear in the population will increase. This is simply a modern way of defining natural selection: the alteration of gene frequencies in the gene pool as a result of differential reproduction. In our discussion of random genetic drift, we mentioned that certain genes make individuals more "fit." This means that these genes give their owners a greater-than-average opportunity to pass them on to the next generation by (1) improving their chances of surviving through the reproductory period, (2) increasing their

attractiveness to potential mates, or (3) improving their chances of raising their family to maturity. In any or all of these situations, the union of gametes is not the random process described by the Hardy-Weinberg law, but certain genotypes have a definite "edge" over others. Thus change in the gene pool does occur. This is evolution—evolution by Darwin's natural selection.

32.7 THE EFFECTS OF INCREASED SELECTION PRESSURE

The vigor with which the forces of natural selection operate varies from time to time and place to place. Man is not the only species that undergoes both "hard times" and "easy times." When times are hard, the forces of natural selection operate with greatest efficiency. We describe this as increased selection pressure. It arises from one or more of several causes. An increase in the number or efficiency of its predators challenges the existence of a species. More frequently, perhaps, its existence may be threatened by other species that compete with it for food, nesting areas, sunshine and water (in the case of plants), or some other essential aspect of its environment. If the population of the species is large, its members are also thrown into direct competition with each other for the necessities of life. Furthermore, a crowded population is more susceptible to parasitism by some other species, and a devastating epidemic may occur. An increase in the severity of any or all of these conditions constitutes an increase in selection pressure. The result of increased selection pressure is a reduction in the variability of the species. Predators and parasites will weed out the weak, the poorly camouflaged, or those who in any other respect are less well adapted to their environment. Only those genotypes in the population that confer the best camouflage, the most effective offensive or defensive weapons, the most efficient food-gathering structures, the greatest resistance to disease, etc., will perpetuate themselves successfully. The deviant, less efficient genotypes will be the first to suffer from increased selection pressure and will become less common.

Increased selection pressure acts to stabilize gene pools

Specialization leads to increased efficiency

Changes in the "standard" genotype may also occur, but they will be in the direction of increased efficiency at withstanding the increased selection pressure. This usually means that the species becomes more narrowly specialized in its requirements and hence more narrowly specialized in its structural, physiological, and behavioral adaptations. The species comes to occupy an ever-narrower portion of its habitat but exploits its diminished share more effectively. The moth with the 10-inch proboscis which pollinates the tropical orchid with the 10-inch nectary has certainly followed this evolutionary trend. Most parasites also show an extreme degree of specialization in a very limited environment (Fig. 16.2).

The final outcome of evolutionary specialization is extinction

Such an evolutionary path may provide temporary success for the species and a resulting increase in the size of the population. If the trend to increased specialization continues, however, the species commits itself to a rather uncertain future. You can well imagine what would happen to the moth with the 10-inch proboscis if its orchid should succumb to

environmental changes. The study of paleontology reveals a large number of extinct animals whose fossil remains suggest that they were extremely specialized. In most cases, we can only speculate as to what environmental change led to their downfall. The principle seems clear, though. Any species that becomes very uniform and increasingly efficient at exploiting an ever-narrower aspect of its environment runs the risk of extinction when its environment changes. Evolution in times of rigorous selection pressure is thus a conservative force. The optimum genotypes are selected for, and less efficient genotypes have a reduced chance of survival.

32.8 THE EFFECTS OF RELAXED SELECTION PRESSURE

The evolutionary picture changes when selection pressure is relaxed. When times are easy, a species can increase its population without at the same time becoming more specialized.

Decreased selection pressure permits increased variability in the population

Reduction in the severity of predation or parasitism or an increase in available food enable deviant genotypes to compete on more nearly equal terms with their better-adapted cousins. A sudden population explosion may occur and with it a marked rise in the variability within the species. History tells us that human societies develop their most flourishing culture in times of prosperity. So, too, relaxed selection pressure enables other populations to experiment with new, perhaps inefficient, genotypes. Instead of being a conservative force, evolution now becomes a dynamic force and the genetic variability permitted by sexual reproduction now has a chance to express itself fully.

The evidence of both the past and the present suggests that the most frequent cause of relaxed selection pressure is entry into a new environment which is relatively free of (1) competing species, (2) predators, (3) parasites, or all three. When the ancestor of Darwin's finches reached the Galapagos Islands, it found at most a handful of songbird species established there. With few reptiles or birds of prey and probably no mammals present, predation must have been practically nonexistent, too. Only under such unusual circumstances was a warblerlike and woodpecker-like finch able to evolve. If true warblers or woodpeckers had been present in the islands, we can be sure that their efficiency would have prevented the appearance of competing forms among Darwin's finches.

In more recent times, the introduction of rabbits into Australia and destructive crop insects into this country have provided just two of many dramatic examples of the explosive success a species can achieve when introduced into a new environment where selection pressure is reduced. Unfortunately, there have not been sufficient field studies to prove that reduced selection pressure goes hand in hand with increased intraspecific variability, but there is some evidence to support this.

Two aspects of evolution

The idea of evolution involves two processes. First is the gradual change in genotype and phenotype of a population of living organisms. Usually these changes are adaptive; that is, the organisms become increasingly efficient at exploiting their environment. Second is the formation of new species. Assuming that life has arisen only once on the earth, the 1.2 million known species of microorganisms, plants, and animals living today (not to mention all the species that have become extinct) must have arisen from ancestors that they shared in common. So a theory of evolution must tell us not only how organisms become better adapted to their environment but also how *new* species are produced.

32.9 WHAT IS A SPECIES?

The zoologist Ernst Mayr defines species as groups of actually or potentially interbreeding natural populations which do not interbreed with other such groups even when there is opportunity to do so. We must qualify this somewhat by adding that if on rare occasions breeding between species does take place, the offspring produced are not so fertile and/or efficient as either of the parents. Although a horse and donkey can breed together, the mule resulting is sterile. (In plants, even this restriction sometimes fails to apply if conditions in the habitat have altered. Plant hybrids *may* be more successful than either parent in such areas.)

It seems quite clear that the process of gradual evolutionary change in a population and the process of species formation are related. Over a period of time, the accumulation of changes in the gene pool of a population must reach a point where we may say that a new species has been formed. This involves a purely arbitrary judgment, however. Who is to say just when the transition was made from one species to the next? Even if we could resurrect some of the ancestral forms to see if they could breed successfully with their modern descendants, our question would remain unanswered. An unbroken line of forms stretches back in time from each living species, and the breaking up of this line into distinct species is an entirely arbitrary (although useful) operation.

A "cline through time"

Evolution has not, however, been just a matter of gradual change in a *single* genealogical line. The fossil record tells us that there has been a marked expansion in the number of species present on the earth. To put this another way, we believe that all our present species have diverged from common ancestors and initially from a single, first form of life. What can the theory of evolution tell us about **speciation,** that is, the formation of many species from few?

Speciation

32.10 THE ROLE OF ISOLATION

The first requirement for speciation is reduced selection pressure and the intraspecific variability it promotes. But this is only the start of the story.

Even with increased variability in a species, there is a continuous intergrading of traits from one extreme to the other. The formation of two or more species from one requires that gaps appear in the population where no intermediate forms exist. For this to occur, some form of geographical isolation of the various subpopulations seems essential. Only then can natural selection or perhaps random genetic drift produce a definite shift away from the gene frequencies of the parental type. It is no accident that the various races or subspecies of living organisms almost never occupy the same territory. The seven distinct phenotypes (and thus genotypes) associated with the seven American subspecies of the Yellowthroat *Geothlypis trichas* (Fig. 32.4) would quickly merge into one if these subspecies occupied the same territory and interbred with one another. Only in isolation can distinctive genetic differences become established.

Subspecies may someday become full-fledged species

Darwin's finches illustrate this point nicely. Not only did the absence of competitors and predators on the Galapagos Islands provide a greatly relaxed selection pressure for the ancestral bird that reached them, but the relative isolation of the various islands permitted the establishment of unique island races or subspecies. From these have arisen the thirteen species which inhabit the islands today (Fig. 32.5). Many of these species are now, in turn, made up of several distinct subspecies isolated on the various islands.



1. Maryland Yellowthroat, *Geothlypis trichas trichas*
2. Northern Yellowthroat, *G. t. brachidactyla*
3. Athens Yellowthroat, *G. t. typhicola*
4. Florida Yellowthroat, *G. t. ignota*
5. Western Yellowthroat, *G. t. occidentalis*
6. Salt Marsh Yellowthroat, *G. t. sinuosa*
7. Tule Yellowthroat, *G. t. scirpicola*

Fig. 32.4 Geographical distribution of the seven subspecies of *Geothlypis trichas*. As a general rule, two subspecies of a single species do not breed in the same territory.



Fig. 32.5 Darwin's finches. The finches numbered 1–7 are ground finches. They seek their food on the ground or in the low shrubs. The finches numbered 8–13 are tree finches. They live primarily on insects. 1. Larger cactus ground finch. 2. Large ground finch (*Geospiza magnirostris*). 3. Medium ground finch (*G. fortis*). 4. Cactus ground finch. 5. Sharp-beaked ground finch. 6. Small ground finch (*G. fuliginosa*). 7. Woodpecker finch. 8. Vegetarian tree finch. 9. Large insectivorous tree finch (*Camarhynchus pauper*). 10. Large insectivorous tree finch (*Camarhynchus psittacula*). 11. Small insectivorous tree finch. 12. Warbler finch. 13. Mangrove finch. (From Biological Sciences Curriculum Study, *Biological Science: Molecules to Man,* Houghton Mifflin Co., 1963.)

The importance of geographical isolation in the formation of species is vividly illustrated by the single finch species that is found on Cocos Island, some 500 miles to the northeast of the Galapagos. The ancestral bird arriving there must also have found a very relaxed selection pressure with few predators or competitors. How different the outcome here than in the Galapagos, though! One species gave rise to thirteen in the various Galapagos Islands, but no such divergence has occurred on Cocos Island. One finch species is still all that exists there.

The distribution of cottontail rabbits in this country provides still another example of the role played by geographical isolation in the formation of species. In the eastern half of our country, only eight species are found, while in the mountainous western states there are 23. In this case, high mountains have provided geographical isolation as effective as the ocean water between the various Galapagos Islands.

32.11 REUNION

We have learned that consistent, distinctive differences in phenotype (and thus genotype) are found within subpopulations of a species that are somewhat isolated from one another. The island races of some of Darwin's finches and the races of *Geothlypis trichas* are cases in point. So long as these forms remain geographically separate, we prefer to consider them different subspecies of the same species. If brought together, they might not, in fact, breed together, but so long as we lack this information, we assume that they would. The question of their status, if they ever do come to occupy the same territory again, is soon answered. If successful interbreeding

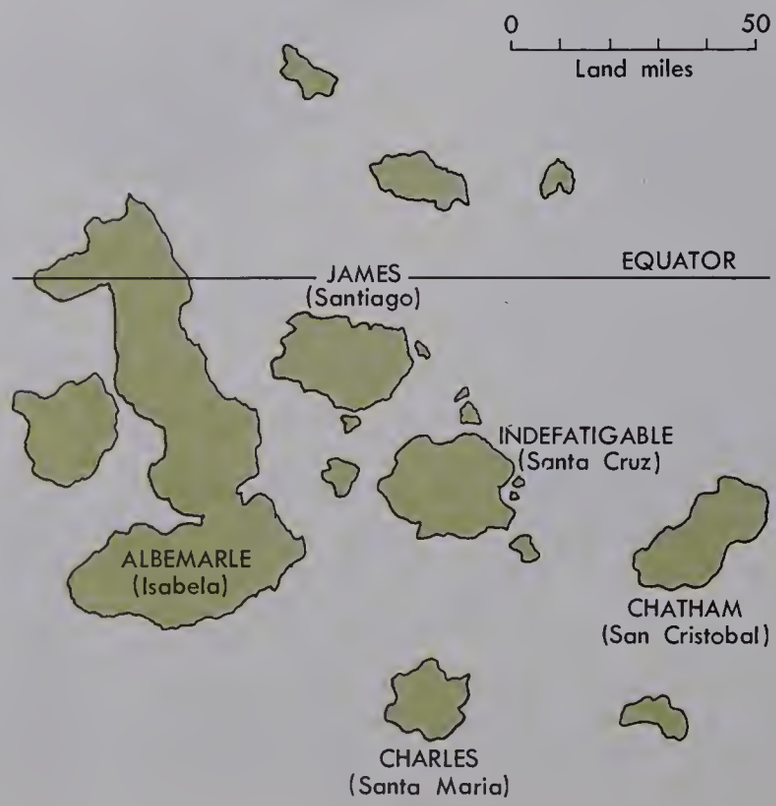


Fig. 32.6 The Galapagos Islands. Now belonging to Ecuador, each island has been renamed. The modern names are shown in parentheses.

If two subpopulations fail to interbreed when given the opportunity, speciation has occurred

occurs, the differences will gradually disappear, and a single population with continuously intergrading traits will be formed again. Speciation will not have occurred. If, on the other hand, the two reunited subpopulations do not interbreed successfully, then speciation will have occurred.

This is well illustrated by the large insect-eating, tree-dwelling finches on the Galapagos, *Camarhynchus pauper* and *Camarhynchus psittacula.* Of the two, *C. pauper* appears to be more primitive in its traits and thus the earlier form. Today it is found only on Charles Island (Fig. 32.6). The closely related *C. psittacula* is found on all the central islands, including Charles. Were it not for its presence on Charles, both forms would be considered subspecies of the same species. Because they do coexist and maintain their separate identity on Charles, we know that speciation has occurred. It looks as though *C. psittacula* evolved in isolation from *C. pauper* to the point where merging of the two groups did not occur when *C. psittacula* recolonized Charles Island.

Nongeographical barriers to interbreeding

What might keep two subpopulations from interbreeding when reunited geographically as *C. psittacula* and *C. pauper* have been on Charles Island? At first it may simply be a matter of one group's lacking the releasers to evoke mating behavior in the other. On Charles, *C. psittacula* has a longer beak than *C. pauper* and the evidence indicates that Darwin's finches choose their mates primarily on the basis of beak size. In other birds, plumage may be the big factor.

In the course of time, other factors that tend to prevent interbreeding may arise. The two groups may come to occupy different habitats in the same area and thus fail to meet at breeding time. The timing of reproductory activity in the two groups may not be the same. The northern and southern forms of *Rana pipiens* could not breed in nature because the females lay eggs at different times of the year. Differences in time of flowering may also prevent otherwise closely related species of angiosperms from interbreeding. Structural differences in the sex organs may also be an isolating mechanism. With continued separation, a final, irrevocable separation of the two groups takes place. This occurs when their genetic make-up becomes so different that no vigorous, fertile offspring can be produced even if mating should occur. At this point, all would agree that absolute speciation had occurred.

Reunion after speciation increases selection pressure

This evolutionary process may be hastened when two formerly isolated groups are reunited. Even if they no longer interbreed, they probably are still similar in many ways, including their requirements for the necessities of life. Thus the reuniting of the two groups may create an intense selection pressure, so intense in fact, that one species is eliminated entirely. Perhaps this has happened to *C. pauper* on some of the central Galapagos Islands. On the other hand, increased selection pressure may, as we have seen, lead to increased specialization within each group and so lessen the competition between them.

The range in beak sizes of *C. pauper* on Charles and *C. psittacula* on Albemarle is roughly the same. This probably means that these two groups take the same type and size of insect. On Charles, however, *C. psittacula*

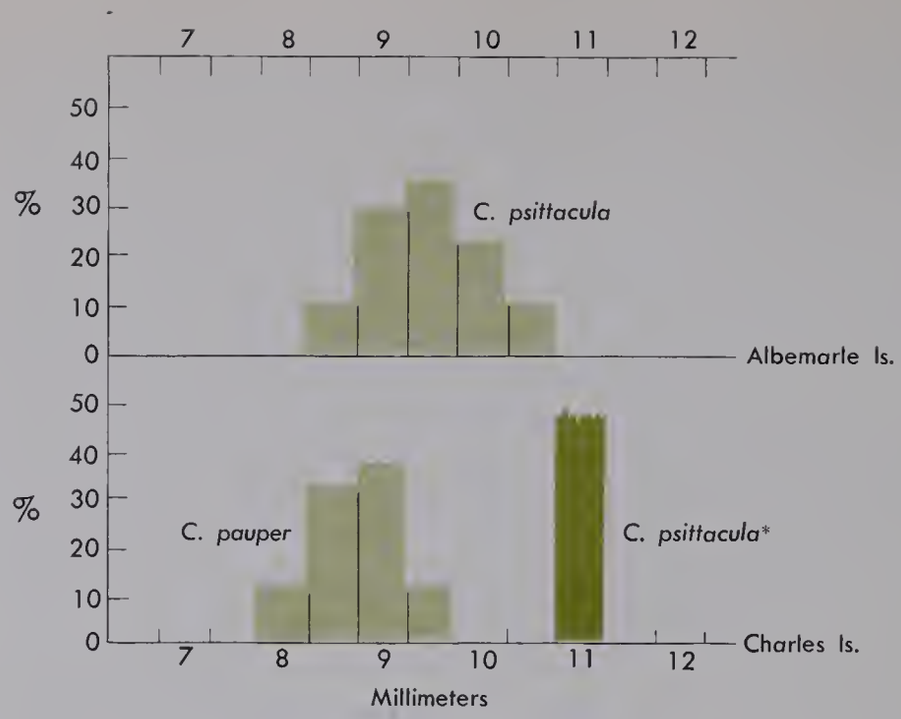


Fig. 32.7 Histograms of depth of beak in *C. pauper* and *C. psittacula*. (Redrawn by permission from David Lack, *Darwin's Finches*, Cambridge University Press, 1947.)

* The number af callected specimens—16— was taa small ta compute reliable percentages, but all fell within the size range indicated.

has a substantially larger beak than *C. pauper* and thus presumably differs enough in its food requirements for the two species to coexist there (Fig. 32.7).

It would be rash to conclude from the study of Darwin's finches that speciation in all organisms has occurred in exactly the same way. But his finches do illustrate beautifully what are probably the absolute essentials for the process. They are:

The steps that lead to speciation

1. Reduced selection pressure (in this case achieved by moving into a new, unoccupied territory).
2. Increased variability within the species (arising from the process of sexual reproduction, working with genes no longer being selected against).
3. Isolation of a subpopulation of the species so that interbreeding with the main stock is prevented. This produces geographic races or subspecies.
4. Reuniting of the isolated group with the parental stock but without the resumption of interbreeding. At this point, true speciation has occurred.
5. Competition between the two reunited forms may be so intense that further divergence in their various traits is hastened.

This process need not occur simply once. Indeed, in the case of Darwin's finches, we are sure that it has been repeated several times, giving rise to new species, which gradually have divided the available habitats between them. From the first arrival have come a variety of ground-feeding and tree-feeding finches including a warblerlike finch and the extraordinary, tool-using, woodpeckerlike species (Fig. 32.5). The produc-

tion of a number of diverse species from a single ancestral one is often referred to as **adaptive radiation.** We shall study several other well-illustrated examples in later chapters.

32.12 SPECIATION BY POLYPLOIDY

Speciation, as we have described it, is a gradual process. It begins with differences between individuals in a population, proceeds to the accumulation of consistent differences found at the subspecies level, and ends with the fixed differences associated with species formation. The entire procedure may take hundreds or thousands of years to be completed. There is one kind of species formation, however, which occurs much more rapidly. This arises from the chromosomal aberration of polyploidy (see Section 18.4).

Polyploidy provides instantaneous reproductive isolation

Meiosis in polyploid (for example, $4n$) individuals produces gametes ($2n$) that are fertile with one another but often not with gametes of the parental type (n). Thus polyploid individuals are immediately isolated genetically from the parental type and a new species has been created almost overnight. In 1927, the Russian plant geneticist Karpechenko reported the artificial production of a plant containing a full ($2n$) set of radish chromosomes and a full ($2n$) set of cabbage chromosomes produced as a result of crossing these two species (which are from different genera). Ordinarily, hybrids of such different parents would be sterile since their gametes would contain only a partial assortment of the chromosomes from each of the two parents. However, Karpechenko's hybrid being polyploid, each gamete contained one of each of the chromosomes found in both the radish and the cabbage ancestors. Fertilization produced vigorous plants which combined traits of both cabbages and radishes (unfortunately, the roots of the cabbage and the leaves of the radish). These plants were capable of breeding successfully with one another but not with either ancestral type. Karpechenko had thus produced in a very brief period a new species of plant. There is abundant evidence that species production by polyploidy, with or without hybridization, has often occurred naturally in plants. The phenomenon is, however, very rare in animals.

32.13 CONVERGENT EVOLUTION

We have noted that species (e.g. bird and bat, whale and fish, woodchuck and wombat) are sometimes found which resemble each other superficially but fail to reveal the homologies that would indicate close kinship. Evolution as a result of which two species of different genealogy come to resemble one another closely is termed *convergent evolution.* It can be explained on the basis of the same forces of natural selection working in a similar way on two originally different phenotypes. There are certain structural and physiological requirements that must be met before any organism, no matter what its ancestry, can fly or swim.



Flying phalanger



Flying squirrel

Wombat



Woodchuck



Fig. 32.8 Convergent evolution. The flying squirrel (top right) and woodchuck (bottom right) are more closely related to you than they are to the marsupials on the left. (This woodchuck, or groundhog, is a rare albino form.) (Courtesy of the New York Zoological Society.)

Convergent evolution is in no sense the opposite of speciation. While two unrelated species may come to resemble one another closely as similar selective forces work on them, each species is, at the same time, diverging from its own ancestral stock. The many Australian marsupials which resemble placental mammals in both appearance and habits (Fig. 32.8) illustrate convergent evolution with respect to the placental mammals. With respect to the ancestral marsupials, however, they represent a most dramatic example of adaptive radiation, in other words, the multiplication of species.

EXERCISES AND PROBLEMS

1. The Kaibab squirrel has long ears and a white belly and lives on the north rim of the Grand Canyon. The Abert squirrel, which lives on the south rim, is quite similar in appearance although it has a black belly.
   a) How can you account for the presence of these two similar, but distinguishable, populations?
   b) What possible futures might await these squirrels if the canyon should be obliterated?
2. Fossil evidence indicates that evolutionary changes in honeybees have taken place unusually slowly. Can you think of a possible reason why natural selection might operate particularly slowly in this group?
3. A large proportion of the so-called "living fossils" is found in the sea. Can you think of a reason for this?
4. How can you account for the superficial resemblance of a porpoise and a fish?
5. What term would Darwin have applied to nonrandom reproduction?
6. List three mechanisms by which variant genotypes are produced in nature.
7. Which of these is denied to a hermaphroditic organism that practices self-fertilization exclusively?
8. What phenomenon must occur for the mechanisms in Question 6 to be effective in producing variant genotypes?
9. The proportion of homozygous recessives of a certain trait in a large population is 0.09. Assuming that the gene pool is in equilibrium and all genotypes are equally successful in reproduction, what proportion of heterozygous individuals would you expect to find in the population?
10. Why are most mutations harmful?
11. Considering the strong selection pressure against the gene for hemophilia, what reasons can you give for the continuing presence of the gene in the human population?
12. What is the genetic effect of continued inbreeding in a population?
13. Why does continued inbreeding often lower the vigor of the individuals in a population?
14. Define a species.
15. How can the Darwinian theory of evolution account for the long neck of the giraffe? How does this differ from Lamarck's explanation?
16. Distinguish between interspecific and intraspecific competition.

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33

THE STRUGGLE FOR EXISTENCE

The Growth of Populations

The Capacity of the Environment

Predation

Parasitism

Defenses against Parasites

Treatment of Disease

The Evolution of Parasitism

Symbiosis

Intraspecific Regulation of Population Size

The Human Population

The Balance of Nature

What Does the Future Hold?

Adaptations ameliorate the struggle for existence

The struggle for existence, in which each species must continually engage, has a number of aspects. These were mentioned briefly in the last chapter. In this chapter we shall examine in greater detail some of the forms this struggle takes. We shall find that the evolutionary mechanisms discussed in the last chapter have enabled species to develop in ways that reduce the severity of the struggle. These developments—structural, physiological, and behavioral—are **adaptations.** Taken together they enhance the ability of a species to survive in its corner of the biosphere.

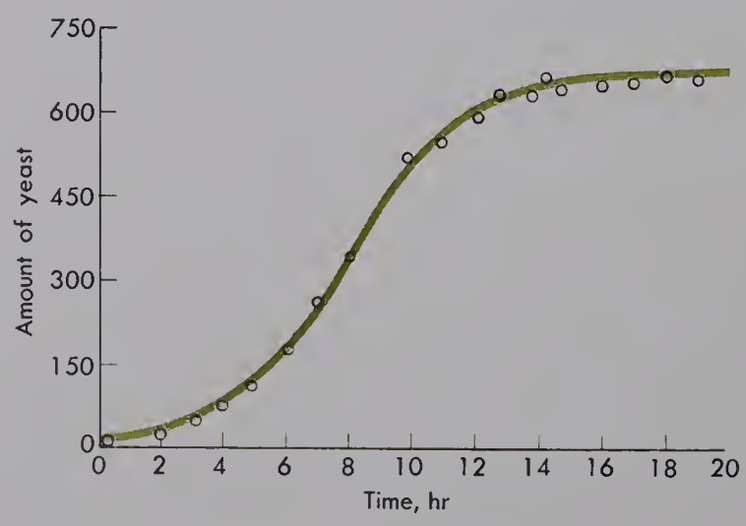
33.1 THE GROWTH OF POPULATIONS

The reproductive potential of all species is logarithmic

There is not a species of living thing that could not completely fill its habitat with its kind if unlimited food were available to it, and if the other physical and biotic factors in its environment were favorable. Some would do it more rapidly than others. The ability of certain bacteria (e.g. *E. coli*) to double their weight and divide every 20 minutes would result in their blanketing the earth in a few days if nothing stopped them. Humans reproduce much more slowly but they, too, could theoretically cover the earth in a surprisingly short period of time. In fact, any species whose couples produce more than two offspring during their lifetime will expand in numbers unless the excess die before they themselves undertake parenthood. Under ideal conditions, then, populations grow logarithmically (Fig. 33.1). As in the growth of individuals, this is simply a reflection of the fact that the product of growth itself grows. The time scale varies from species to species, but the potentiality for logarithmic growth is present in them all.

Checks on population growth

The growth curve of individuals is *S*-shaped. After the logarithmic or exponential period, the rate of growth declines and, in many cases, growth finally ceases. Luckily, the same phenomenon also occurs in the growth of populations. Physical and biotic factors in the environment eventually check further population growth. This check arises from either or both of two factors. (1) The **capacity of the environment** to supply the needs of



Growth of population of yeast cells

Fig. 33.1 The S-shaped curve of growth.

the species is limited. A point is always reached when the amount of food or, in the case of plants, sunlight, water, and minerals is insufficient to support any further increase in the population. This is not just a matter of an absolute limit to the resources available; it also involves the fact that other species are competing for these same necessities of life. (2) The efforts of **predators and parasites** to satisfy their need for food also provide definite checks on the size of populations. As a population increases, there are predators and parasites ready and willing to harvest it for their own use. These two factors, then, working to keep each species from realizing its potential to overwhelm the earth, serve to maintain the "balance of nature." Let us see just how each one operates.

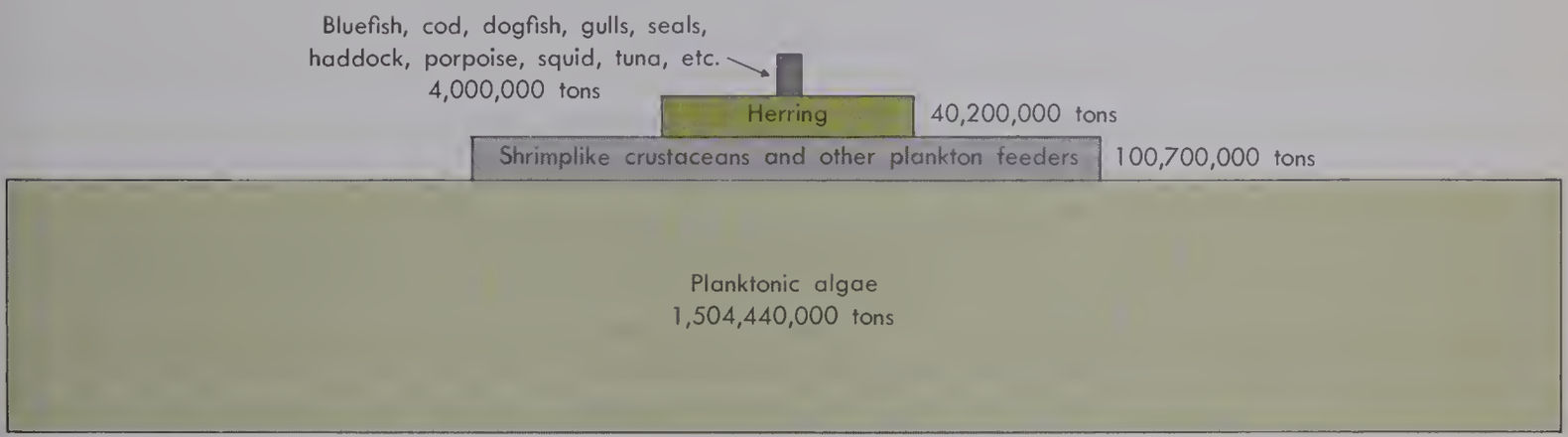


Fig. 33.2 A pyramid of mass in the open sea. (Adapted from Lorus J. Milne and Margery Milne, *The Biotic World and Man,* 2nd. ed., Prentice-Hall, Inc., 1958.)

33.2 THE CAPACITY OF THE ENVIRONMENT

The capacity of the environment of a given species depends ultimately on the amount of energy that species can trap. In Chapter 10 we examined the flow of energy through populations, beginning with autotrophs and ending with heterotrophs. We also learned of the sharp losses of energy that occur as it flows from one link to another in a food chain. These losses establish strict limits on the biomass of populations at each trophic level (Fig. 33.2).

The ecological niche

Some animals consume a variety of foods in their diet (look back at Fig. 10.5). Other animals are more selective in their food. The cotton boll weevil feeds exclusively on cotton plants. The term **niche** is often used to describe the particular role that an organism plays in the community, especially its role as a consumer of food. The niche of the hawk is a fairly broad one. It alters its diet in accordance with the relative abundance of the large variety of animals that can serve as prey. The niche of the cotton boll weevil is a narrow one. Where there is no cotton, there are no boll weevils. The occurrence of similar niches in different parts of the world helps explain the phenomenon of convergent evolution. The flying pha-

langer and wombat occupy in Australia the niches which are filled in North America by the flying squirrel and woodchuck (Fig. 32.8).

While the niches of many animal species in a community overlap, it may well be that no two species ever occupy *exactly* the same niche in the same location. If they did, we would expect that one species would be more efficient than the other at exploiting the niche and would eventually replace the less well-adapted species completely. This principle of **competitive exclusion** may not always hold. For example, two species of insects might occupy the same niche but other factors (weather, parasitism, predation) could be so severe that neither population could become large enough to diminish the food supply seriously. Generally, however, a close study of the feeding habits of two species which seem to occupy the same niche reveals some differences. While three of Darwin's finches, *Geospiza magnirostris, G. fortis,* and *G. fuliginosa* (see Fig. 32.5), eat a certain amount of the same food, each includes in its diet seeds of sizes not usually consumed by the others. We can thus conclude that the number of niches occupied by heterotrophs in any region is approximately equal to the number of species there.

Competitive exclusion

The limited capacity of the environment to furnish energy establishes an absolute limit to population size. However, this limit is not apt to be reached under natural conditions. Every source of food is exploited by more than one species. Grasshoppers, rabbits, and cows all compete for the available grass. Thus, the population of each is kept in check by competition with the others. Under these conditions, we can appreciate that any inherited traits which diminish the severity of interspecific competition will, by natural selection, tend to become established in the population. The usual outcome is the evolution of adaptations which increase the feeding efficiency of the species. Increased efficiency goes hand in hand with increased specialization, however, and the result is a progressive narrowing of the niche of each species. The cotton boll weevil and the moth with the 10-inch proboscis each represent an extreme of feeding specialization. Their niche is narrow but they exploit it more efficiently than their competitors can.

Interspecific competition

Plants, too, are thrown into competition with other plant species for sunlight, soil, water, and minerals. Many are the specialized adaptations by which plants reduce interspecific competition for these necessities. Species with shallow root systems are able to coexist with deep-rooted species because each is tapping a different region in the soil. The seeds of shade-tolerant species fail to germinate in sunny locations where they would be at a competitive disadvantage and, similarly, the seeds of shade-intolerant species will not develop successfully in shady locations. In the desert, the leaves shed by the brittlebush leave a poison in the soil that keeps competing annuals at their distance. The evolution of epiphytism, vines, and (in the case of many woodland shrubs) growth before the forest trees reach full-leaf, represent adaptations to compete more effectively for sunlight.

33.3 PREDATION

The second major check on the size of populations is predation. The majority of heterotrophic species secure their food by preying on other organisms. Although exceptions exist, most predators are larger than the prey they consume. Their relationship with their prey is usually a temporary one, just long enough to consume it or at least part of it. Animals which prey upon other animals almost always kill their prey. On the other hand, herbivorous predators (e.g. deer, rabbits, insects) usually just browse on a portion of the prey's body. The regenerative ability of most green plants then ensures that additional food will be available at a later time.

When we consider the central role food plays in the lives of all animals, we should not be surprised at the many adaptations which increase the effectiveness of predation. Think of the eyes of the eagle, the sharp ears of the owl, the keen sense of smell of the wolf, the echo-location of the bat, and the value of wings and legs to predators. Strong jaws, cutting teeth, sharp claws, hooked beaks, the long tongue of the frog, the fangs of the rattlesnake, and the mandibles of insects are other examples of structural modifications which assist predation.

Defenses against predation

On the opposite side of the coin, these devices are also useful to avoid being eaten. Eyes, ears, and a keen sense of smell are just as effective at discovering predators as prey. The ability to run, fly, or swim away from a predator when it is detected is naturally of great survival value. It is not the only solution to the problem, though. Camouflage is another way of minimizing attack by predators (Fig. 33.3). The inky smokescreen expelled by the startled squid hides its escape route. It has been shown that the



Fig. 33.3 Ptarmigan in summer plumage, incubating her eggs. In the winter these birds are white. (Courtesy of the American Museum of Natural History.)

robber fly is not so apt to be eaten by an animal previously exposed to the well-armed bumblebee (see Fig. 31.17). Weapons such as teeth and jaws, a sting, poison fangs, and the pincer legs of the crayfish can be used defensively as well as offensively. The quills of the porcupine, and the scent glands of the skunk are other familiar examples. Millipedes secrete poisonous hydrocyanic acid when disturbed. Many insects have special glands and aiming devices which enable them to squirt potential predators with such noxious substances as 85% acetic acid and 40% formic acid. Some animals are often able to escape from predators by sacrificing a portion of their body. Grasp a "glass snake" (really a legless lizard) by the tail, and in an instant you will be holding the tail only while the former owner goes off to regenerate a new one. There are crabs, in fact, that when molested by a predator (an otter, for example), grab it with a pincer claw, detach the claw, and scuttle away leaving the predator to cope with its new acquisition. Many a sea cucumber has escaped consumption by expelling its sticky, offensive-smelling viscera when molested.

Intraspecific cooperation may reduce predation

Cooperation between members of the same species often reduces the severity of predation. A mother bobwhite fluttering away from her nest while feigning a broken wing, distracts the attention of a predator from her young. Grazing ungulates are usually organized so that the strong are on the outside of the herd, the weak within. The presence of many sets of eyes and ears in the herd makes the predator more easily detected and his approach more difficult. Smelt, a kind of fish, have been shown to release a pheromone into the water when alarmed. This serves to warn the other members of the school. When a honeybee stings an enemy, she releases a chemical, isoamyl acetate, which excites other bees to join the attack.

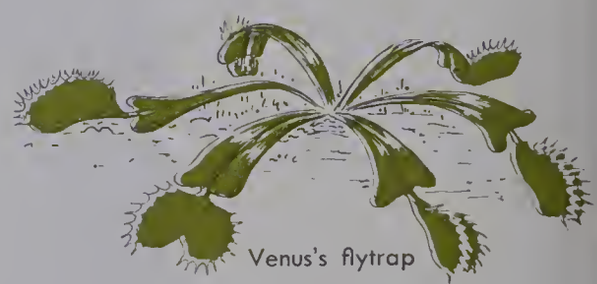


Fig. 33.4 Rapid turgor movements enable the modified leaf tips to trap insects. Digestion of the prey presumably makes nitrogen available to the plant, thus compensating for the mineral-deficient, boggy soils in which these plants live.

Insectivorous plants

The only plants that are predacious are the insectivorous plants. They have interesting modifications for trapping and digesting insects. Specialized leaves of the Venus's flytrap (Fig. 33.4) close quickly (because of a sudden loss of turgor) when an insect alights on the inner surfaces. The animal is then digested by enzymes.

Is there any explanation for this curious reversal of behavior in which a plant eats an animal? All the insectivorous plants contain chlorophyll and thus, like other green plants, they are able to manufacture their carbohydrates by photosynthesis. To manufacture proteins, however, green plants require nitrates. The activity of the nitrifying bacteria provides one major

source of these. These bacteria convert ammonia (produced when dead organisms decay) into nitrates, which can then be absorbed by green plants for protein synthesis. But nitrifying bacteria cannot operate successfully in an environment that is very acid. Bog soils, with a pH of four or even less, are quite acid and it is in just such soil that the insectivorous plants are found. Insect eating is probably an adaptation to secure nitrogen compounds when the normal source is limited.

More common among plants are devices to avoid predation. Toxic chemicals (including hydrocyanic acid) and thorns or spines are defensive mechanisms frequently found. The ability of grasses to repeatedly send up new leaves from underground enables them to withstand a degree of browsing which would be fatal to most dicots.

Interdependence of predators and prey

The balance between prey and predator is a delicate one and somewhat self-regulating. An increase in the population of the victim permits the predator population to increase. Increased predation then lowers the population density of the prey species and is followed by a reduction in the concentration of predators. Of course, that opens the way for another build-up in the prey population, and so it goes. There is apt to be some lag in these shifts with the result that the populations of predators and prey not only fluctuate, but the periods of maximum population of the two species do not always coincide. Figure 33.5 illustrates the cyclical relationship between the population estimates for the varying hare ("snowshoe rabbit") and for its chief predator, the lynx, in the region around Hudson Bay in Canada.

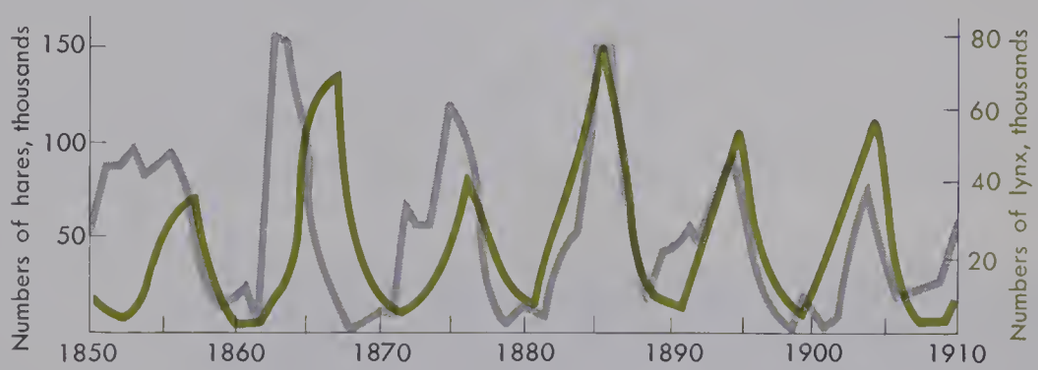


Fig. 33.5 Cyclical fluctuations of the varying hare and lynx populations in the Hudson Bay region from 1850 to 1910.

It is obviously important to the balance of nature for the rise and fall of predator and prey populations not to be so extreme that the prey are completely exterminated. If this should occur, the predator population would soon follow suit unless an alternate source of food was available. This might be impossible for a predator occupying a narrow niche. In a restricted habitat such as a small freshwater pond, mutual extinction could thus be a real threat. We know practically nothing as yet about how this danger is generally avoided in such situations, but at least one fascinating control mechanism has recently been discovered. In the eastern United States, freshwater ponds are often populated by tiny mosquito fish (*Gambusia*), which feed on mosquito larvae (thus serving a useful function for

us). *Gambusia* is, in turn, preyed upon by the pickerel, a fast-swimming, voracious predator. Mosquito fish do not hide in underwater vegetation and, after watching a hungry pickerel at work, it seems miraculous that any mosquito fish could escape being eaten by it. Escape they do, thanks to a nice, self-protective mechanism. Whenever mosquito fish detect the presence of a pickerel, they move up to the surface of the water and begin splashing about. So long as this goes on, the pickerel makes little effort to attack them and, when it does attack, often misses. Let one member drift beneath the surface, though, and he is devoured in an instant. The signal to the mosquito fish is a chemical one. When water where pickerel have been swimming is added to an aquarium containing mosquito fish, they immediately carry out their self-protective response. It is not yet fully understood just why the pickerel is so often unsuccessful while its prey is at the surface, but the value of this mechanism is perfectly clear. By failing to exterminate its prey, the pickerel avoids exterminating itself. By simply cropping the surplus *Gambusia* population, both populations are maintained at a stable level.

It is also easy to see how natural selection maintains this response in *Gambusia.* Any individuals in the population whose genotype leads to a diminished response will be the first ones to disappear, genotype and all, down the throat of the pickerel.

33.4 PARASITISM

Parasitism vs. predation

A parasite is an organism that lives on or in the body of another organism (the host) from whose tissues it derives its nourishment and to whom it does some degree of damage. The distinction between parasites and predators is not always clear. The tick or leech attaches itself to the body of its host for a short period while it sucks blood. In a sense, this is simply a form of predation in which the smaller and weaker organism is browsing on the larger and stronger one. In the case of blood-sucking hookworms, the relationship is of long duration and clearly one of parasitism.

Although there are obvious exceptions, a parasite usually does not kill its host. To do so is to deprive itself of a meal-ticket. Instead, the well-adapted parasite consumes just enough host tissue to supply its own needs without destroying the host. It has been said that a parasite lives on the host's income while a predator lives off the host's capital.

Diversity of parasites

Almost all parasites are relatively small invertebrates or microorganisms. Mites, fleas and lice, hookworms, flukes, and tapeworms are representative parasites from each of three animal phyla. Many protozoans are parasitic. The role these latter organisms play in disease as well as that played by the bacteria and fungi was unknown until the 19th century. Louis Pasteur is justly honored as the man who first showed that many diseases of man and other animals are caused by invading bacteria. The last major category of infectious agents to be discovered were the viruses. The first of these, the tobacco mosaic virus (TMV) was discovered in 1892 by the Russian scientist Iwanowsky.

There is probably not an organism on this earth that is not parasitized at some time during its life. Animals are attacked by representatives of every category of parasite. Plants are parasitized by nematodes, fungi, bacteria, viruses, and a few other plants. Even microorganisms have parasites; bacteria, for example, are attacked by viruses (bacteriophages).

Almost all locations on the host's body may become occupied by parasites. Viruses, some bacteria, and the malarial parasites invade and multiply right within the very cells of the host. Most parasitic bacteria and protozoans, as well as many roundworms and flukes, invade the tissues of their host. In the case of animal hosts, one finds tapeworms, roundworms, protozoans, and bacteria within the alimentary canal. Most insect parasites (e.g. the louse) are ectoparasites, that is, they live on the surface of the body.

How parasites invade their hosts

One problem faced by all parasites is how to invade the host. For animals (including man), four routes are used. Many parasites (e.g. tapeworm cysts, typhoid bacilli) are ingested in food or water. Many bacteria and viruses gain entrance to the body by being inhaled. These may cause respiratory tract diseases (e.g. "colds"). During coughing or sneezing, parasite-laden droplets are discharged into the air and can readily be inhaled by others (Fig. 33.6). Copulation enables the microorganisms causing venereal disease to gain entrance into the body of a new host. This occurs in other animals as well as man. Finally, insect and tick bites often act as hypodermic needles, introducing protozoans, bacteria, and viruses into the body of the host. Plants are often inoculated with disease-causing viruses as a result of the bites of aphids and other sucking insects.

Once the parasite has gained entrance into the body of the host, there are three possible outcomes. The host may be killed by the parasite, it may tolerate it for an indefinite period of time, or it may destroy the parasite completely. Which of these three possible outcomes actually happens is



Fig. 33.6 A violent, unstifled sneeze. High-speed flash illumination reveals the cloud of droplets produced. These are a major factor in the spread of bacteria and viruses that enter the body by way of the respiratory tract. (Courtesy of Dr. Marshall W. Jennison, *Scientific Monthly,* Vol. 52, pp. 24–33, Jan., 1941.)

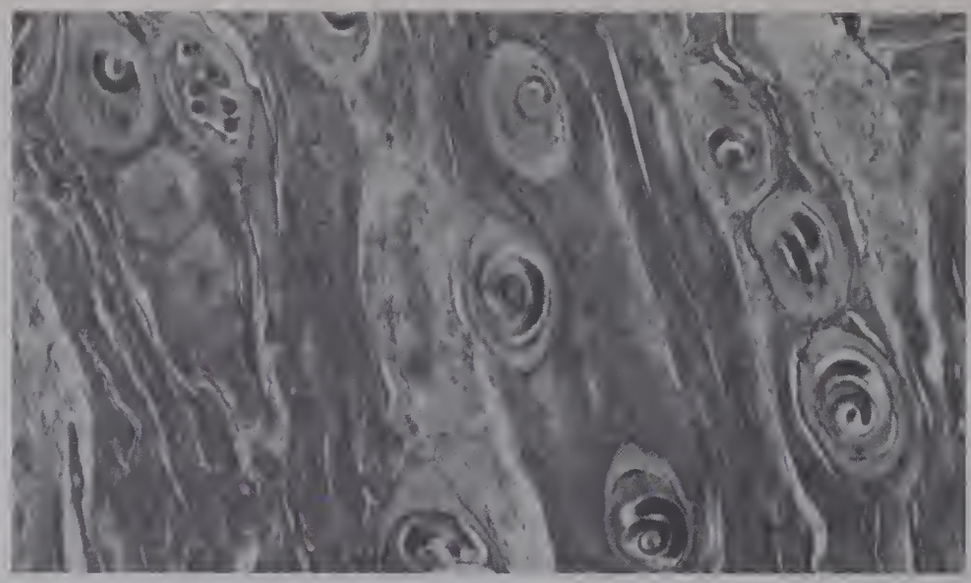


Fig. 33.7 Larvae of the parasitic nematode *Trichinella spiralis* encysted in pig muscle (19 X). Ingestion of undercooked pork containing these parasites produces trichinosis.

dependent upon four factors: (1) the number of invading parasites, (2) their nature, (3) the defenses of the host and, where man is involved, (4) the nature of any treatment given.

Size of invasion

Ingestion of undercooked pork containing a few *Trichinella* cysts (Fig. 33.7) usually causes such a mild case of trichinosis that it is not even diagnosed as such. Eating heavily infected meat frequently results in death. Often the body copes successfully with small numbers of invading parasites but succumbs to heavier infestations. Of course the absolute number of parasites which will overwhelm the host varies with the severity of the damage caused by the particular parasite.

Pathogenicity

Parasites do vary in the severity of the damage they do to the host. Man may harbor a tapeworm for years with little harm done. However, 80% of the infections by a certain strain of rickettsias causing Rocky Mountain spotted fever end in death if no treatment is given.

Parasites damage their host in two ways. One is by actually consuming its tissues. Hookworms, parasitic amoebas, and the malarial parasites all cause this sort of damage. Some parasites do not consume enough of the material of their host to be dangerous, but in the process of their metabolism they release toxins which poison the host. The bacteria that cause tetanus ("lockjaw"), diphtheria, and scarlet fever are dangerous for this reason.

Toxins

Tetanus toxin interferes with synaptic transmission in the central nervous system. Diphtheria toxin exerts its poisonous effect on the protein-synthesizing machinery of the cell. A curious twist to this kind of damage has been discovered in certain fungi which parasitize insects. These fungi produce juvenile hormone and, in so doing, upset the normal pattern of metamorphosis. In this case, the damage is not produced by a poisonous toxin but by the secretion of a normal constituent of the host in abnormal amounts at abnormal times.

What happens when a parasite enters its host also depends on the defensive mechanisms that the host puts into operation to combat the invasion. For obvious reasons, we know more about man in this respect, so he will be our example.

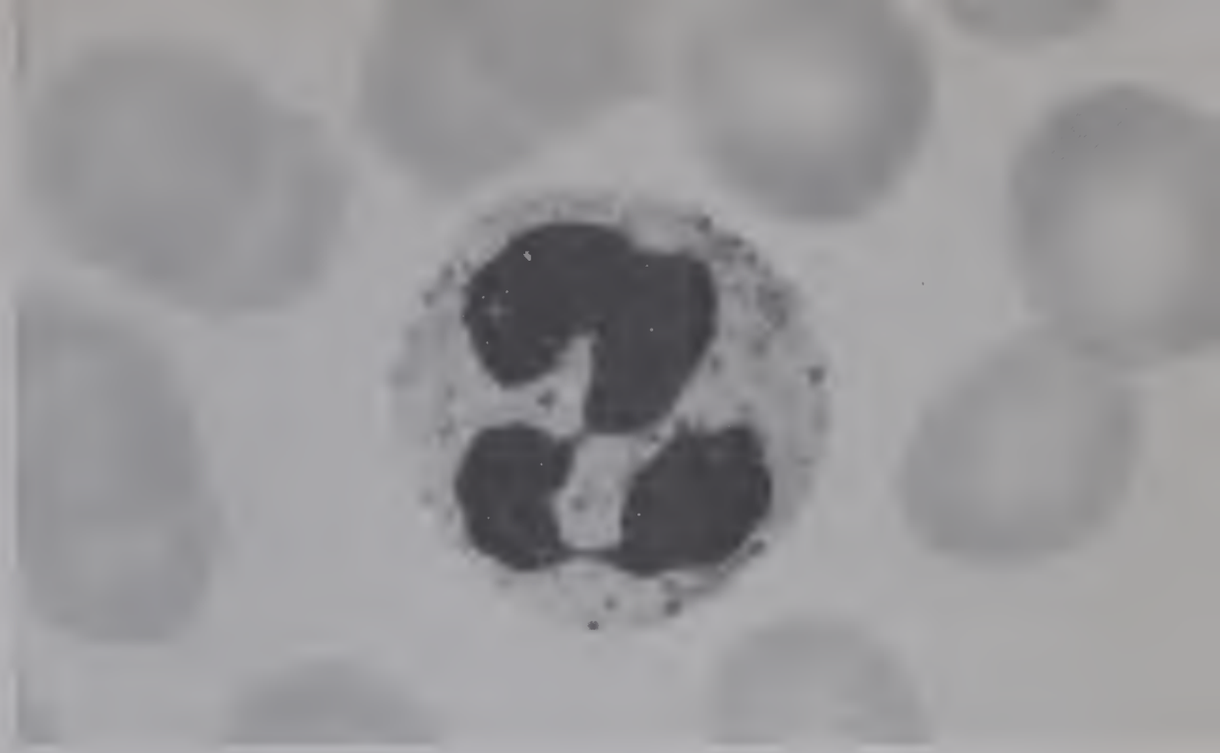


Fig. 33.8 Neutrophil, a phagocyte, in human blood, 2000 X. (Courtesy of CCM: General Biological, Inc.)

33.5 DEFENSES AGAINST PARASITES

Let us examine the lines of defense that are brought into play when bacteria, carried on a splinter, are introduced beneath the skin.

Mechanical damage by the splinter will kill some cells and as these disintegrate, they provide nourishment for the bacteria which were introduced into the wound. These begin to grow and multiply. However, damaged cells also liberate substances which signal the body to begin taking action against the invader. One of these, histamine, causes precapillary sphincters in the area to relax, thus increasing the blood supply. The walls of the capillaries become "leakier." The result of these two actions is an accumulation of lymph in the tissue spaces. This increase is not drained away by the lymph capillaries as they become plugged with a mesh of fibrin. Histamine also stimulates the conversion of a serum globulin into polypeptide *kinins* (not to be confused with the cytokinins of plants), which pass into the tissue spaces and accelerate these changes. Some substance, perhaps a kinin, is produced which attracts **phagocytes** from the blood stream. These, mostly neutrophils (Fig. 33.8) migrate through the capillary walls into the tissue spaces and begin ingesting the bacteria and damaged cells. After consuming a number of bacteria, they die, and their disintegrated bodies are one component of pus. Other phagocytes establish a living barrier between the region of tissue destruction and intact healthy tissue. In this way, they isolate the site of infection.

Inflammation

All of these localized responses constitute **acute inflammation.** The external symptoms are redness (caused by the increased blood supply), tenderness (caused by the kinins), and if the wound is open, the oozing of pus. If all goes well, the process of acute inflammation will prevent the infection from spreading, and after all the bacteria have been destroyed, healing can begin.

It should be noted, though, that an inflamed tissue cannot properly carry out its normal functions. If the inflammation becomes widespread, it may become more dangerous than the parasite itself.

Furthermore, even localized inflammation sometimes fails to contain the invading parasites. They may escape into the lymph and/or blood capillaries. A second line of defense comes into play at this time. The sinuses of the **lymph nodes** are lined with "fixed" phagocytes, which engulf bacteria passing through and thus prevent them from reaching the bloodstream. If the invasion is especially heavy, the lymph nodes may themselves become infected and swollen. Hidden infections are sometimes first detected by the appearance of these "swollen glands" in such areas as the neck, armpits, and groin.

Role of the lymph nodes

Liver and spleen

Bacteria that reach the bloodstream are engulfed by fixed phagocytes which line the sinuses of the **spleen.** The blood sinuses in the **liver** are similarly equipped to screen out the steady stream of bacteria which penetrate the lining of the intestine and reach the liver by way of the hepatic portal veins. In this way, these organisms are kept from entering the general circulation.

The production of antibodies

For many infections, the thing that finally tips the balance in favor of the host is the production of **antibodies.** And as we saw in Chapter 30, the ability to produce a *secondary response* may knock down a reinvasion so quickly that we never develop symptoms. In this way, immunity to that parasite is acquired.

Interferon

It has been known for some time that people sick with one virus possess a temporary immunity against infection by other viruses. This protection occurs because cells attacked by the first virus produce an antivirus agent called **interferon.** Interferon is a protein molecule produced by mammalian cells shortly after infection and, in every case, faster than antibodies can be produced. Unlike antibodies, interferon is not specific; that is, it is a single substance which seems to be effective against all viruses. In some way it alters the ribosomes of the cell so that they do not read the virus messenger RNA properly, and therefore new virus particles cannot be manufactured. If safe ways can be found to enhance the body's production of interferon, it should provide an excellent weapon against virus diseases (which are just the ones against which other drugs are so ineffective).

We have been discussing the ways in which the human body responds to invasion by parasites. Although man has been far more extensively studied in this respect than any other organism, there is mounting evidence that we are not unique in our responses. Interferon is produced by other mammals and also by birds. Antibody formation occurs in all vertebrates, and there is some evidence that even invertebrates possess a similar defensive mechanism. Phagocytosis is found throughout the animals; in fact, it was first observed in the transparent body of the microscopic water "flea" *Daphnia* (a crustacean). Plants, too, seem to possess mechanisms for defense against parasites, although very little is known as yet about them.

33.6 TREATMENT OF DISEASE

Man differs from the other animals in being able to take supplementary action to assist the natural processes of his body in combating disease. In fact, in the last half century, he has been so successful at this that infectious disease no longer plays a major role in deaths before old age, at least in the more prosperous parts of the world. When considering this miraculous achievement in applied biology, it is somewhat startling to realize with what a small number of weapons man is able to combat parasites directly. These weapons are: (1) a limited number of chemical compounds whose origin, in most cases, has been in the organic chemistry laboratory, (2) the antibiotics, and (3) the antisera. All the rest of the vast number of substances that fill the druggist's shelves make the patient feel better but have no direct action whatsoever on the parasite causing his ailment.

1. Chemotherapy. A moment's thought should make it clear why there are so few substances capable of combating man's parasites once they are within his body. Throughout this book, we have stressed the underlying similarity in cell structure and biochemistry of all organisms, including man and his parasites. There are many substances which, when administered to man, would kill his parasites, but unfortunately they would kill him, too. The problem is to find substances that act against the parasite without damaging the host seriously.

Histologists (biologists who study tissues) have known for years that some biological stains are quite selective in their action. They will stain certain types of cells and not others. Following this clue, the German bacteriologist Paul Ehrlich attempted to find a substance which would combine selectively with the spirochete that causes syphilis and, in so doing, kill it without killing the host. After a long patient search, he found, on his 606th attempt, a substance which did just this. Thus was born (in 1910) Salvarsan, the first chemotherapeutic agent synthesized by man.

Another German scientist, Gerhard Domagk, followed his lead and, in 1932, found that a red dye called prontosil was effective against the streptococci ravaging his daughter's body. Later, a French chemist discovered that all the activity of prontosil resides in just a portion of the molecule. This portion (which is not a dye at all) was **sulfanilamide,** the first of a

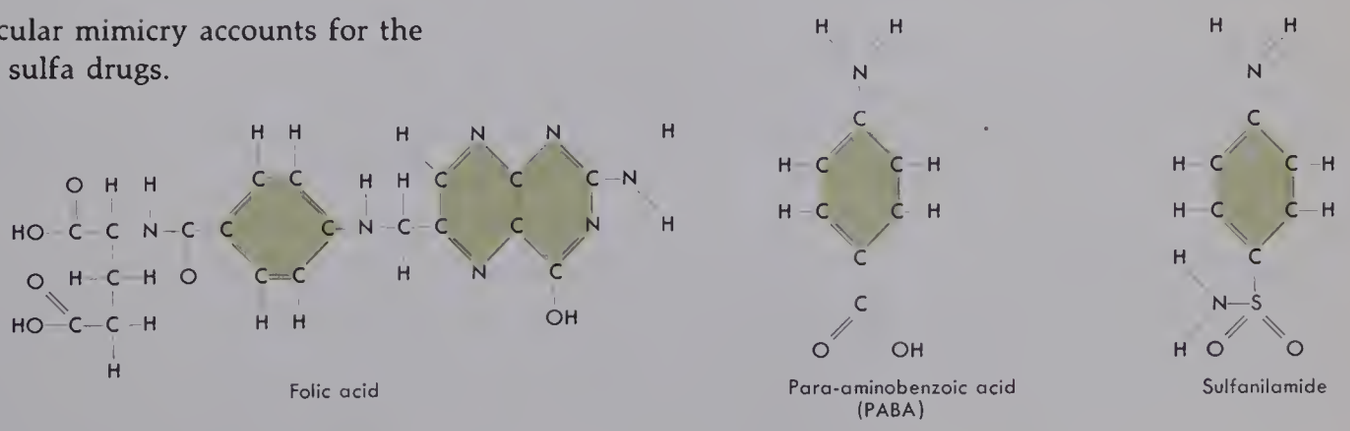


Fig. 33.9 Molecular mimicry accounts for the effectiveness of sulfa drugs.

The sulfa drugs

large family of sulfa drugs, which were developed just in time to play a major role in reducing the number of casualties in World War II.

The action of the sulfa drugs is dependent upon molecular mimicry. All organisms require folic acid (one of our B-vitamins) in order to grow. Most bacteria are able to synthesize folic acid from simpler building blocks, one of which is para-aminobenzoic acid (PABA). A glance at Fig. 33.9 shows the similarity between the PABA and sulfanilamide molecules. When an excess of sulfanilamide is present in the medium, the bacteria use it instead of PABA but, in so doing, fail to produce the folic acid they must have. If we need folic acid, too, why isn't sulfanilamide lethal to us as it is to the bacteria? The answer is that the folic acid molecule is a vitamin for us; that is, we cannot synthesize it. Here, then, is a biochemical difference between host and parasite which can be exploited in killing one without killing the other.

Although the discovery and exploitation of such differences has been slow, some of man's metazoan and protozoan parasites can now be eliminated chemically. Hookworms and tapeworms are anesthetized and then expelled with the aid of a laxative. Atabrine and a few other drugs have proven of great value in curing malarial infections. One by one, effective chemical weapons are being developed against other parasitic protozoans. With rare exceptions, though, virus infections must still be overcome by the body's own defenses. For direct attack against bacterial infections we are generally limited to the sulfas and the antibiotics.

Antibiotics interfere with an essential metabolic process that occurs in the parasite but not in the host

2. Antibiotics. There is no fundamental difference between the antibiotics and the chemotherapeutic drugs discussed above. In both cases, specific molecules are involved, and laboratory synthesis of antibiotics is often possible, too. However, each antibiotic was discovered originally as the secretion of a fungus or a bacterium, which inhibited the growth of other bacteria. The first, and in many ways still the best, antibiotic was penicillin. Its discovery stemmed from the observation by Alexander Fleming that bacterial growth was inhibited in a culture dish which had accidentally become contaminated with a mold of the genus *Penicillium* (Fig. 33.10). The significance of this discovery was appreciated in time to develop techniques of mass production of penicillin for use in World War II. Since then, laboratories throughout the world have tested hundreds of thousands of specimens of fungi and bacteria for antibiotic activity. When such has been found, the substance has been tested for safety. Very few antibiotics have passed all the hurdles, but chloramphenicol (sold as "chloromycetin"), streptomycin, the tetracyclines ("aureomycin" and "terramycin"), and a few others have now joined penicillin in the fight against bacterial infections.

Passive immunization

3. Antisera. Antiserum is serum which contains antibodies against one or more specific antigens. If injected into the body of a person recently infected by or exposed to the antigen, it confers an immediate, but temporary, immunity on him. This immunity is described as passive because it is not produced by the patient's own immunological mechanism.



Fig. 33.10 The growth of bacteria on the agar in this culture dish has been inhibited near the three circular colonies of the fungus *Penicillium notatum.* The antibiotic penicillin, diffusing outward from the colonies, is responsible for this effect. (Courtesy of Merck & Co., Inc.)

Antiserum is manufactured in live animals. For example, if sublethal quantities of tetanus toxin are injected into a horse or sheep, the animal develops **antitoxin.** By bleeding the animal periodically and extracting its serum, the antitoxin can be harvested and used to treat human patients who may have been infected with tetanus bacilli from a splinter or other wound. Unfortunately, there is some danger to the use of antisera prepared in the bodies of other animals, because we can become allergic to other foreign proteins present in the mixture. Therefore, it is far wiser to use tetanus toxoid to develop one's own active immunity, although this must be done before infection actually occurs.

The gamma-globulin fraction of adult human blood is apt to contain antibodies against a number of common disease organisms. For this reason, it is often separated from donated blood and used as an antiserum. Temporary protection for children or adults exposed to hepatitis or measles is commonly achieved by injections of gamma-globulin pooled from many blood donors.

Man has greatly increased his life expectancy by learning how to aid his own body in ridding itself of parasites. If improperly administered, however, these techniques involve some dangers. For example, prolonged exposure to low doses of an antibiotic will alter the pressure of natural selection and actually select for those spontaneous mutants capable of withstanding the drug. Staphylococci that have developed resistance to penicillin are now a serious problem in many hospitals. It may seem ironical that this threat has arisen right within the hospital, center of man's fight against disease, but it should not surprise any student of the mechanism of evolution.

33.7 THE EVOLUTION OF PARASITISM

Mutual adaptation of parasite and host

Parasitism, like predation, is a two-way relationship. It is the product of evolutionary adaptations on the part of both host and parasite. The fact that each species of parasite is restricted to one or, at most, a few species of hosts suggests this. The extreme specialization of some parasites to conditions in their host and the host's ability to tolerate their presence is further evidence of a long history of mutual adaptations.

The significance of mutual adaptations between host and parasite is often nicely demonstrated when a parasite gains accidental entrance into an abnormal host. Larval tapeworms, hookworms, and flukes, whose normal host is some vertebrate other than man, nonetheless may occasionally invade man. When they do so, they migrate widely through the body, causing extensive damage as they go. It is almost as though they were hunting for normal conditions. The rickettsia that causes Rocky Mountain spotted fever and the virus that causes yellow fever are two examples of parasites which exist in animal "reservoirs" (rodents and monkeys, respectively) and do little damage to these hosts. The outcome of their entry into man is often quite a different story.

There is no reason to think that mutual evolutionary change on the part of parasite and host ever ceases. As the host develops increased resistance, his parasites are apt to develop counterweapons. We see this process hastened through man's intervention. The use of antibiotics has been countered by the evolution of antibiotic-resistant bacteria. The development of rust-resistant grains by selective breeding is inevitably followed by the appearance of mutant rust strains capable of parasitizing them. One of the major and never-ending tasks of plant breeders in the U.S. Department of Agriculture is to keep one step ahead of the rusts and ensure that

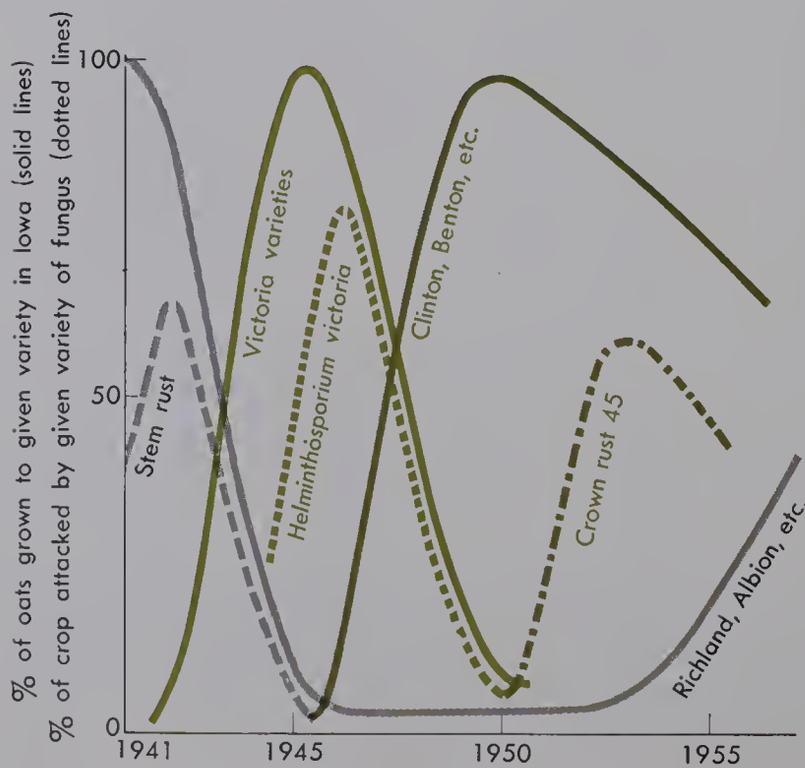


Fig. 33.11 Host-parasite relations between the domestic oat (*Avena sativa*) and some of its parasites.

grain farmers will always be able to switch to a still newer resistant variety as the older varieties begin to succumb. Figure 33.11 traces the changing tides of battle between our domestic oat and the fungi that parasitize it.

The risks of monoculture: the southern corn leaf blight

In 1970 the United States witnessed a vivid demonstration of the ability of parasites to co-evolve with their host. Prior to 1970, the southern corn leaf blight, caused by the fungus *Helminthosporium maydis,* had been a relatively minor problem. The hybrid corn varieties in use were relatively resistant to the organism, although they were known to be susceptible to a *Helminthosporium* race found in the Philippines.

In 1969 corn losses due to *Helminthosporium* began to rise in Florida, presumably because of the fresh appearance of a mutant race having infective properties similar to the Philippines race. During the 1970 season, this new race moved out of Florida, swept through the Gulf states, and then moved on into the Midwest and eventually into Canada. In some areas, 50% or more of the crop was lost. Overall, the U.S. corn harvest was reduced 15%—a loss of approximately one billion dollars.

Fortunately, plant breeders had already developed corn strains resistant to another fungus and these turned out to be resistant to the new race of *Helminthosporium* also. However, the lessons are clear: the development of host resistance is always temporary and the more widespread the cultivation of a genetically uniform crop, the greater the risk of disastrous epidemics.

Even when the mortality caused by a parasite is low, the size of its population may wax and wane. This occurs when an epidemic leaves all survivors immune to the parasite. Not until that immunity is reduced or a new crop of nonimmune youngsters comes along will the density of susceptible individuals reach a point where another epidemic can occur. Figure 33.12, which shows the annual incidence of measles in South Australia from 1916 through 1920, illustrates this nicely.

Are parasites degenerate?

Parasites are often referred to as degenerate. In one sense, this is true. During the course of adapting to conditions in their particular niche, parasites have lost structures essential to the welfare of their free-living relatives. Looked at from the parasite's point of view, though, the loss of organs that are no longer appropriate represents a gain in efficiency and, thus, improved specialization. The tapeworm has no eyes, no digestive

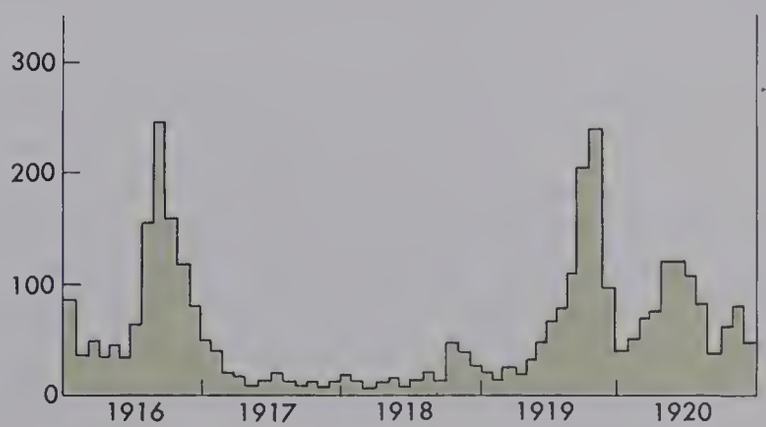


Fig. 33.12 Monthly notifications of measles in South Australia from 1916 to 1920. (Redrawn by permission from Sir Macfarlane Burnet, *Natural History of Infectious Disease,* 2nd ed., Cambridge University Press, 1953.)

tract, and only vestiges of nervous, excretory, and muscular systems. But what good would these be anyway in the human intestine? On the other hand, the tapeworm has a scolex, a cuticle resistant to digestive enzymes, a highly efficient reproductory system, and a shape which practically ensures that it will not accidentally obstruct the intestine and endanger its host.

Loss of nonuseful structures and functions is characteristic of all parasites. *Rafflesia* is a parasitic angiosperm found in Malaya, which does not have roots, stems or leaves. It does, however, have tubes which penetrate the tissues of its host and one of the largest flowers (3 to 5 feet in diameter) known. This extreme emphasis on reproduction is also found in *Sacculina*, a barnacle which parasitizes crabs. The adult consists of no more than a sack (hence the name) containing reproductory organs, and not until the larvae were identified could it even be determined that the organism was a crustacean.

Viruses seem to represent an extreme of degeneration. The smaller viruses have none of the enzyme systems necessary for a free life. They consist simply of (1) DNA (sometimes RNA) capable of instructing the host cell how to manufacture more virus and (2) a coat of protein which helps the virus invade its host.

How parasites transfer from host to host

A serious problem facing all parasites is how to get from one host to another. Exit from the host body can be made by way of the lungs (during coughing and sneezing), by way of the anus (in the feces) and, in a few cases (e.g. the spirochete that causes syphilis), directly through the skin and mucus membranes. Bacteria and viruses that cause infection of the air passages and lungs are examples of the first case. They leave by the same route they entered. Intestinal parasites such as bacteria, viruses, and protozoans, leave in the feces or, in the case of tapeworms, hookworms, etc., deposit eggs which are expelled in the feces. Where personal and public sanitary conditions are poor, there is a good possibility that some of these organisms will be ingested by other hosts. Some of the worms which inhabit our alimentary canal produce larval stages which must develop in a second, intermediate, host before they are capable of infecting man again. The **intermediate host** of the pig tapeworm (*Taenia solium*) is the pig. It seems pretty clear that the intermediate host in this case is a device that improves the chances of the parasite's reentering the **primary host,** man. Man is far more likely to ingest undercooked pork than human feces. This is not true for pigs raised under unsanitary conditions. The largest (33 feet or even more) tapeworm of man, the fish tapeworm, requires three hosts in order to complete its life cycle: a freshwater crustacean (*Cyclops*—Fig. 33.13), fish, and man. When you consider that man's feces often reach bodies of water, that freshwater fish eat *Cyclops* and man may eat improperly cooked fish, it is easy to see how this complicated life cycle actually improves the chances of this parasite's getting from one man to another.



Fig. 33.13 *Cyclops,* a microscopic crustacean that can serve as the intermediate host in the life cycle of a tapeworm (22 X).

The problem of transport is even more acute for those parasites that live in our blood or other tissues. The malarial parasite, the yellow fever virus, and the roundworm which causes elephantiasis can pass from man



Fig. 33.14 The sand tiger shark with two remoras attached. The presence of the remoras does not seem to inconvenience the shark, and they benefit from scraps of food left uneaten by their host. (Courtesy of New York Zoological Society.)

Vectors

to man only with the help of an intermediate host, the mosquito, capable of withdrawing the parasite from the blood of one host and inoculating it into the blood of another. We use the term **vector** for any animal (most of them are insects) that transmits pathogenic microorganisms in this way.

Fecundity of parasites

Even with elaborate life cycles that make reentry into the primary host easier, the chances of any one individual parasite's doing so are extremely small. It is therefore not surprising that many of our parasites are amazingly fecund, that is, they produce enormous numbers of offspring. The fish tapeworm, mentioned above, discharges up to one million fertilized eggs into its host's feces each day. A female hookworm may lay 25 to 35 thousand eggs each day throughout her five-year life span. Once we understand the problem a parasite faces in getting from host to host, we can appreciate why so many parasites (e.g. the tapeworms, *Sacculina,* and *Rafflesia*) are little more than efficient machines for sexual reproduction.

33.8 SYMBIOSIS

Parasitism is one form of symbiosis

Symbiosis means "living together." It refers to the situation in which the niche of one species happens to be more-or-less closely associated with the body of another organism. The organism whose niche it is always benefits from the relationship. The organism who supplies the niche may be injured by it, as occurs in parasitism. In many cases, however, the organism that supplies the niche may be relatively unaffected by the relationship. This situation is called **commensalism.**

Commensalism

The relationship between the remora and the shark is a classic example of commensalism. The dorsal fin of the remora is modified into a sucker by means of which the remora can form a temporary attachment to the shark (Fig. 33.14). The shark does not seem to be inconvenienced by this and makes no attempt to prey upon the remora. When the shark does feed, however, the remora is in a position to pick up scraps the shark fails to

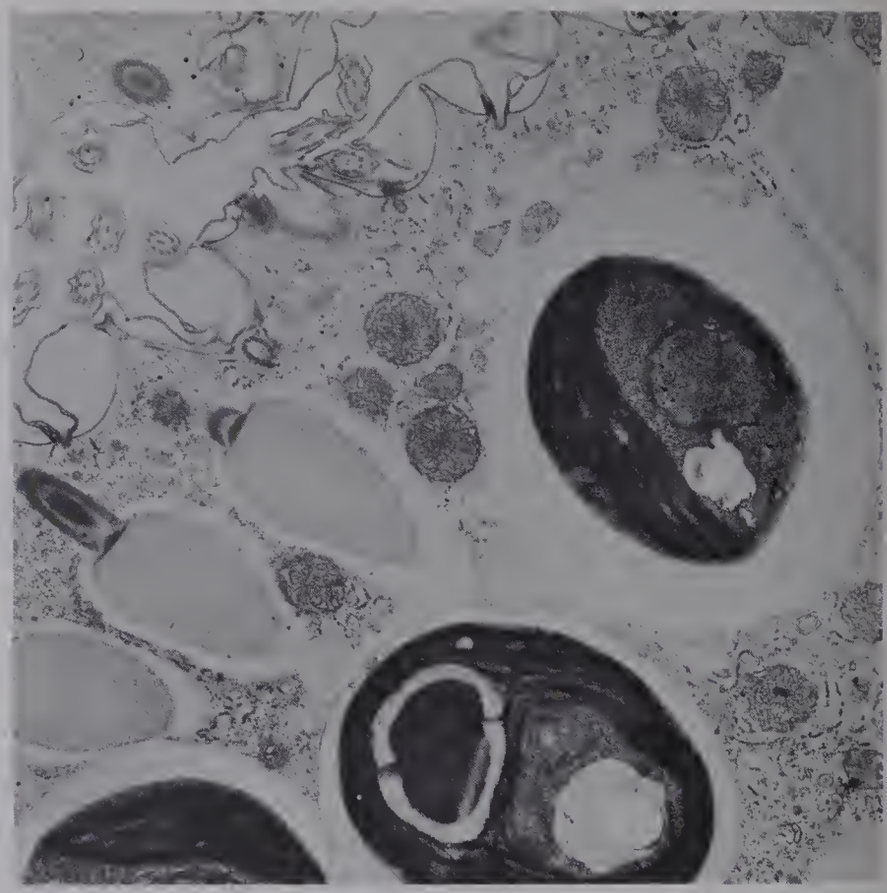


Fig. 33.15 Endosymbiosis. A protozoan, *Paramecium bursaria,* which harbors unicellular green algae (the dark ovals) within its cell. The algae supply food to their host. Both organisms can be cultured apart from each other, but then the paramecium must be given extra food. When brought together again, the paramecium ingests the algae and incorporates them in vacuoles. Many other aquatic heterotrophs, such as certain sponges, planarians, and clams, also harbor algae within their cells. (11,000 X, courtesy of Stephen Karakashian.)

consume. There are certain species of barnacles that are found only as commensals on the jaws of whales. There are even species of barnacles found only as commensals on the barnacles that grow on whales!

Many of the bacteria living in our large intestine should be classed as commensals. They feed on undigested food materials and generally do not harm us. In fact, the experience gained from rearing germ-free laboratory animals suggests that some, at least, of the bacteria that live in the intestine benefit their host. Germ-free animals (e.g. mice) are abnormal in a number of ways, and it is now standard practice to deliberately infect such animals with several species of microorganisms so that the animals will develop normally.

Mutualism

Symbiotic relationships in which *each* species benefits are called mutualistic. (Some biologists prefer to restrict the term symbiosis to such relationships.) The relationship between rhizobia and their legume hosts that we examined in Chapter 2 is an example of **mutualism.** Although each organism is able to survive independently (soil nitrates must then be available to the legume), growth together is clearly beneficial to them both.

Lichens are an example of mutualism between a fungus and an alga. Although the alga may be able to survive alone in nature, the fungal partner is never found to do so. The heterotrophic fungus receives carbohydrates from the alga, while the alga is protected from dehydration by the tough, gelatinous walls of the fungus. Acids secreted by the fungus may also aid the algal partner by liberating minerals from the substrate (e.g. rock).



Fig. 33.16 Indian pipe, a flowering plant that lacks chlorophyll. It gets its nourishment through mycorrhizal fungi that connect its roots to those of a chlorophyll-containing species such as a spruce or pine tree. (Courtesy of J. W. Thompson.)

Algae are often found living within the bodies of heterotrophic organisms (Fig. 33.15). Their presence provides an internal supply of food and oxygen. The former may be sufficient to enable the animal host to go without eating for long periods. The alga benefits by the availability of CO_2, the protected location, and the fact that it can be transported to a spot where ample light is present. The cellulose-digesting bacteria in the cow's rumen provide valuable nutrients to their host in return for a protected, moist, food-filled environment.

Mycorrhiza: mutualistic association of a fungus and a plant root

Mutualistic relations between plants and fungi are very common. The fungus invades and lives in or among the cortex cells of the secondary roots. The association of fungus and root is called a **mycorrhiza.** A number of experiments have demonstrated clearly that the presence of mycorrhizal fungi greatly increases the efficiency with which the host plant absorbs minerals from the soil. Some mycorrhizal fungi also secrete antibiotics which may help protect their host from invasion by parasitic fungi and bacteria.

The advantage of the mycorrhizal relationship to the fungus has also been shown. The fungus gets its nourishment from the sugars that the plant translocates to its roots. In fact, mycorrhizal fungi may establish conduits for nutrients *between* plant species. The colorless, and hence heterotrophic, Indian pipe (Fig. 33.16) secures its nourishment from mycorrhizal fungi that are attached simultaneously to the roots of some autotroph such as a pine or spruce. Radioactive carbon administered to the spruce tree eventually turns up in the Indian pipes, even though they may be growing some distance away on the forest floor.



Fig. 33.17 An ant (*Mycetosoritis hartmanii*) feeding in its fungus garden. The white filaments surrounding the ant are the fungus which is growing on leaf fragments harvested by the ant and placed in its garden. (Courtesy of John C. Moser, United States Forest Service.)

Many of the mushrooms that we find growing on the forest floor are the spore-forming bodies of mycorrhizal fungi. The truffle, prized by gourmets, is always found in oak forests because the fungus that produces it establishes mycorrhiza on oak roots.

Parasitism may evolve into mutualism

Mutualistic relationships may be the evolutionary outcome of what began as parasitism. In 1966, K. W. Jeon discovered a culture of amoebas that had become infected with bacteria (60,000 to 150,000 per cell). The infection affected the amoebas adversely, slowing their rate of growth and making them much more fragile. Five years later, the amoebas still had their bacteria but now no ill effects could be seen. Most interesting of all, the amoebas—or at least their nuclei—had become dependent upon the bacteria. With micromanipulators it is a relatively easy task to remove the nucleus from an amoeba and replace it with another nucleus. When the nucleus was removed from an infected amoeba and replaced with one from a noninfected strain, the new combination worked very well. However, when the nucleus from an infected cell was placed in noninfected cytoplasm, the combination generally failed to survive. Evidently, proper functioning of these nuclei now depended upon some (unknown) contribution from the bacteria. What started as parasitism had become mutualism (the bacteria cannot be grown outside their host).

Mutualistic relationships often involve intricate adaptations of structure, function, and even behavior on the part of the two species. Some species of ants secure their food by growing gardens of a particular fungus in their underground nests (Fig. 33.17). The fungus is nourished by leaves the ants collect, bring underground, and chew into a nutritious pulp. The fact that the fungus is never found except in ant gardens indicates a close

physiological adaptation to the conditions of its culture. The behavior of the ants is certainly adapted to the needs of the mutualistic relationship. Even a special structural adaptation exists: a pouch in the head of the queen into which she stuffs some of the fungus before going off to start a new colony! Figure 33.18 illustrates still another kind of mutualistic relationship that involves elaborate behavioral adaptations.

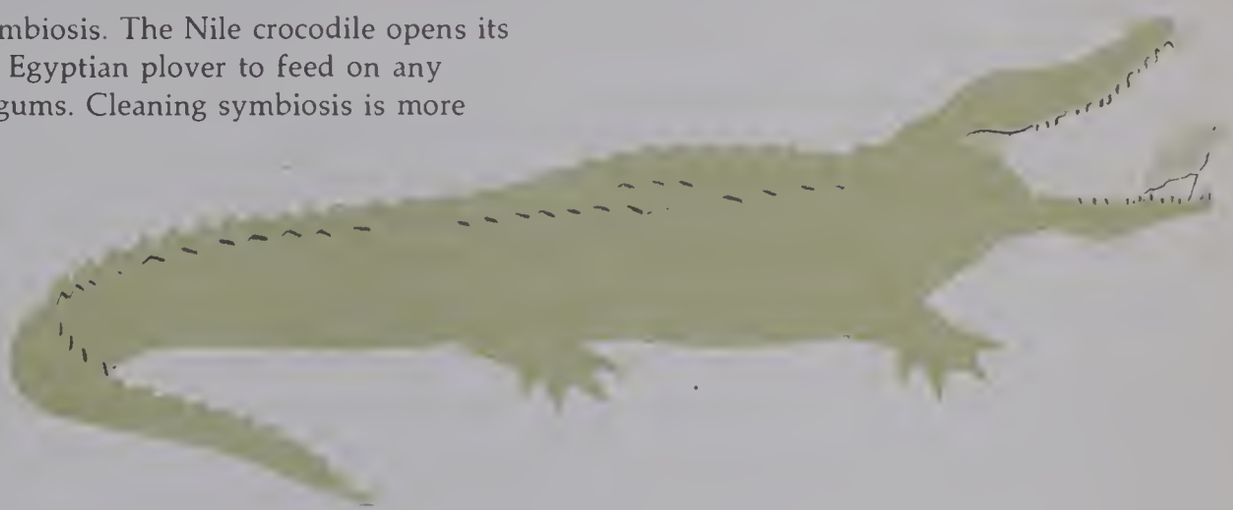


Fig. 33.18 Cleaning symbiosis. The Nile crocodile opens its mouth and permits the Egyptian plover to feed on any leeches attached to its gums. Cleaning symbiosis is more common in fishes.

33.9 INTRASPECIFIC REGULATION OF POPULATION SIZE

Population growth is dependent on birth rate as well as death rate

Neglecting minor cyclical fluctuations, populations usually remain stable despite the enormous reproductory potential of all living organisms. The control measures are quite effective. Predators and parasites feed upon the surplus. If they fail, the limited quantity of essential materials in the environment, that is, its limited capacity, will prevent an unlimited population increase. On the one hand, there is early death at the hands of predators and parasites. On the other, there is early death from starvation. Early death, then, seems to be the chief mechanism by which population growth is held in check. It need not be the only mechanism. Remember that the growth of a population is dependent on birth rate as well as death rate. Are there mechanisms in nature for reducing the number of young produced in each generation to below the reproductive potential of the species? The answer is yes, three of them.

Birth control in natural populations

1. One way to achieve smaller populations is to reduce the number of offspring produced by each pair of adults. Exactly how this is accomplished among animals other than man is not yet clear. At least one species of free-living flatworms has been shown to produce fewer young as the population of adults in its container increases. This phenomenon, which may be caused by an inhibitory chemical released in the water, occurs even when ample food, oxygen, and water are available. There is good evidence that hydras living in a crowded culture release into the water a substance (probably a protein) that inhibits asexual reproduction. Fruit flies living

under crowded conditions lay fewer eggs. Laboratory rats living in a confined area soon reach a stable population size even though abundant food is available. This comes about through a sharp rise in infant mortality both before and after birth, a special case of early death. Reduced maternal care and even cannibalism take a heavy toll of the newborn.

Breeding territories

2. Another mechanism which limits the number of individuals in the next generation is to limit the number of parents. Some mammals and birds achieve this by establishing breeding territories. Each mating pair occupies an area of a size sufficient to supply all its needs including those of its offspring. One or both members defend this area against intrusion from other members of the same species. Thus, they not only ensure that the capacity of their immediate environment will not be exceeded, but they may also keep the general population in check by preventing breeding among the surplus members of their species.

Honeybees carefully adjust their population size to the food supply

3. The formation of closely regulated societies is a third mechanism of population control which reduces the need for early death. Although there are many worker females in the beehive to accomplish the work of the hive, only the queen has functional reproductory organs. Her rate of egg-laying is adjusted to the over-all needs of the hive. Whenever bad weather or poor flowering reduces the amount of food being brought into the hive, she lays fewer eggs. In the late summer, she ceases her egg-laying entirely and thus avoids the necessity of using the hard-won winter stores of food for the raising of young.

Although each of these devices reduces the number of offspring, each one actually promotes survival of the species as a whole. Competition within the species and with other species for the necessities of life is reduced, and the chances of exceeding the capacity of the environment to support life thereby lessened.

33.10 THE HUMAN POPULATION

Is man, too, governed by the principles we have been discussing? He is a heterotrophic organism dependent ultimately upon the organic molecules produced by plants. He has a sufficiently high reproductory potential to live up to the capacity of the environment. Like all other organisms, he is subject to attack by many parasites. However, if we can safely extrapolate from studies of wild chimpanzee and baboon populations, predation has probably never presented a significant check on his population size (except for man's unique form of intraspecific predation, war). Famine, disease, and war have limited man's population growth in the same way that the populations of other species are kept in check.

Predation plays no significant role in controlling the size of the human population

Man does, however, differ from other organisms in significant ways. His large cerebrum, his ability to use tools, and his language (which permits the accumulation of knowledge) have enabled him to alter his physical and biotic environment far more drastically than any creature which

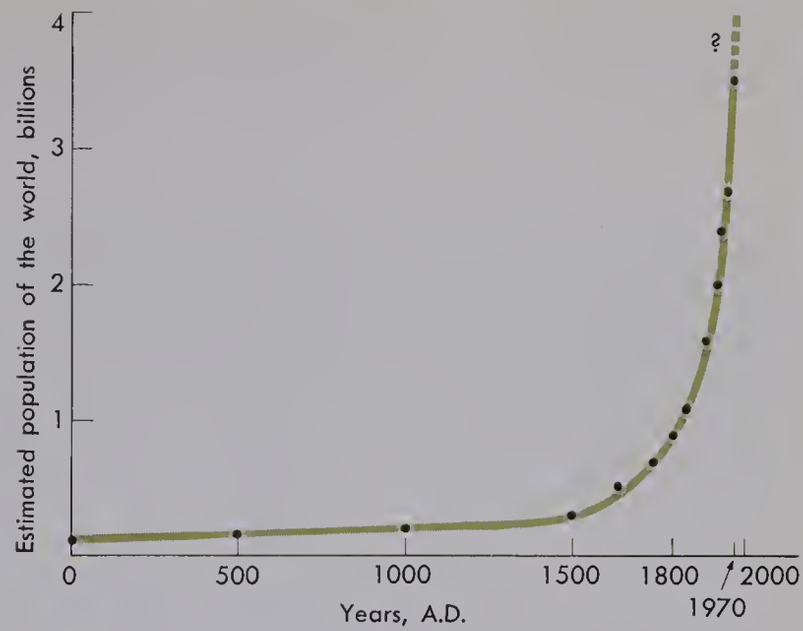


Fig. 33.19 Growth of the human population. The estimates since 1800 are based on more accurate data than those before. This graph has had to be revised for each new edition of this book. Each change has consisted of extending the solid part of the graph to indicate that the projected population estimates in one edition had become realized by the time of the next edition.

The widespread use of public health measures has drastically reduced death rates from infectious disease

has ever gone before. Over the past 100 years he has eliminated, at least in the more prosperous parts of the world, infectious disease as a major cause of early death. The removal of this check on population has resulted in a dramatic increase in the number of humans inhabiting the earth (Fig. 33.19). As we have seen, in other animals the removal of these checks on population would quickly result in the species' expanding to the limit of the environment. Man has so far avoided this in North America, Europe, and a few other areas by improving his agricultural practices as fast as, or faster than, his numbers have increased. Present crop yields are sufficient to (and do) support a human population many times greater than that which could be supported by foraging for food as other animals do.

We have greatly increased the capacity of the environment to feed us

Has man, then, freed himself from the ecological rules that govern other creatures? The answer is no. By his unique and powerful influences on the physical and biotic environment, he has achieved a population that otherwise would have been impossible. The inescapable fact remains, however, that even if he could eliminate all early deaths through parasitism and predation and even if he could exploit autotrophs on every square foot of the planet not occupied by himself, there would still be a limit to the number of humans that could exist. His reproductory potential is sufficient to reach that limit.

Malthus and Darwin knew that no population can ever grow indefinitely. Either an increase in the death rate or a decrease in the birth rate must follow any period of population growth. Some observers, alarmed by the present rapid rise in the world's human population, have advocated the former. War, the withholding of medical assistance, even the deliberate extermination of human populations, have been suggested, and, in some cases, carried out. On the other hand, there are those who prefer to see man deliberately limit the number of offspring produced in order to avoid the necessity for early death from famine, war, or disease. This can be done in a number of different ways, some of which, as we saw in the previous

Other population control measures

section, are used by other animals, e.g. celibacy and inadequate maternal care. Humans have also long relied on certain contraceptive techniques and, as we have seen in earlier chapters, the variety and effectiveness of these is increasing. Nevertheless, there are problems with this approach to population control. The desire to reproduce is as strong in man as in other animals. The religious and social convictions of many are in conflict with any attempt to thwart this desire. There is no third alternative, however. The human population can grow only as large as the capacity of the environment to support it, and, although we have not reached them yet, this capacity has absolute limits.

Even as man's knowledge and skill achieve further increases in the capacity of the environment, every citizen must consider whether it is desirable to have the population increase accordingly. A population living close to the capacity of its environment faces a marginal existence at best. Although the necessities of life may be met one year, crop failure anywhere would, as it often has, lead to widespread famine the next. Furthermore, a population getting just enough food to stay alive and reproduce is not apt to have the interest or leisure to develop and maintain the cultural achievements which distinguish us from the other animals.

It must also be recognized that man's success at increasing the capacity of his environment has sometimes been a short-term affair. He has temporarily increased the productivity of some regions at the expense of their long-term capacity to support life. As with the person living off capital instead of income, a day of reckoning awaits.

33.11 THE BALANCE OF NATURE

In altering ecosystems to provide more resources for himself, man has had profound effects on the population dynamics of many other species. The very act of growing crops drastically alters the balance of nature. A variety of plant species, each with a low population density, is suddenly replaced by acres of a single plant. This man-made population explosion sets the stage for a major build-up in the populations of the insects and parasites which attack that crop. Acres of cotton fields increase enormously the capacity of the boll weevil's environment. Wheat fields stretching as far as the eye can see provide virtually unlimited nourishment for the wheat rust and easy transmission from host to host. The competitors of cultivated plants, the weeds, also flourish where the soil has been exposed and the natural vegetation replaced. Raising herds of domesticated animals, e.g. cattle and sheep, similarly alters the kinds and numbers of other species in the region.

The hazards of monoculture

Man is forced to alter the balance of nature in this way in order to feed himself. He must, however, expend heroic efforts to maintain this unbalanced state. Chemical poisons are one of man's most important weapons in his never-ending battle with the insects over food. Despite the enormous amount of money and effort expended, the insects take about one-tenth of all the food we grow (Fig. 33.20). This represents some four billion

Chemical insecticides



Fig. 33.20 A cornfield before and after invasion by grasshoppers. (Courtesy of U.S.D.A.)

dollars in crop losses each year. If it were not for the use of chemical insecticides, the figure would be far higher, so high, in fact, that we would be transformed from a nation that still enjoys the headache of food surplus to one facing widespread famine.

The development of insecticide resistance

The use of chemical insecticides is not a cure-all. Nature is too adaptable for that. Within a few years of the first use of DDT, insects resistant to it appeared in several parts of the world. This should have been no surprise. Evolution works almost as rapidly in insect populations as it has in the bacterial populations that have developed resistance to penicillin. Fortunately, as in the case of antibiotic resistance, the work of chemists has provided a steady flow of new insecticides which, by affecting other links in the insect's metabolism, has enabled effective control to be maintained.

Lack of selectivity of insecticides

A second drawback to chemical control is that even the most carefully applied insecticides usually kill organisms in addition to the ones intended. Earthworms, fish, crustaceans, snails, and a wide variety of mammals and birds may succumb to poisons designed for certain harmful insects. Valuable insects are often killed, too. Once apple growers learned to control with chemicals the codling moth larva and apple maggot that attack their fruit, they soon found that scale insects and mites began to become a problem for the first time. The reason was simply that the poisons that

killed the codling moth also killed the natural enemies of the scale insects and mites. Without this check on their population, their numbers increased alarmingly until new sprays, especially designed to control them, were applied.

Concentration of pesticides in food chains

The hazard of pesticides to "innocent" organisms is particularly acute for those species that live at the ends of food chains. DDT is not easily broken down or excreted by organisms that ingest it. Consequently, it accumulates in the body, especially in fat deposits. As a result of the spraying of a marsh to control mosquitoes, the microscopic aquatic organisms (the plankton) of the marsh will accumulate traces of DDT. In eating this plankton, small fishes and filter-feeders like clams are, in a real sense, harvesting DDT too. Concentrations of DDT ten times greater than those in the plankton have been measured in clams. Furthermore, the process of concentration continues right up to the end of the food chain. Gulls, which feed upon clams, may accumulate DDT to 40 or more times the concentration in their prey. This represents a 400-fold increase in concentration along the length of this short food chain. While gulls seem to be surviving despite such pesticide burdens, there is abundant evidence that other creatures at the ends of food chains (e.g. ospreys, pelicans, falcons, and eagles) are suffering serious declines in fecundity and hence population because of this phenomenon.

Biological control of pests

An alternative to controlling one pest after another by chemicals is to encourage the natural enemies of the pests. Such biological control methods may be quite effective. Scale insects are preyed upon voraciously by ladybird beetles, and these have been cultured and released for this purpose. Although slower-acting than chemicals, they have provided effective control in some instances. Japanese beetle grubs are now controlled in some areas by treating the soil with the spores of a bacillus which causes a fatal illness in these insects. Another bacillus is being grown commercially so its spores can be used against tent caterpillars, the larvae of the gypsy moth, and other leaf-eating insects. Even weed pests have been attacked biologically. The Klamath weed, introduced into California in 1900, had overrun and ruined some 270,000 acres of valuable range land by 1945. In that year, two species of beetles which, after careful testing, had been shown to eat Klamath weed exclusively, were released in large numbers. Their activity has reduced the Klamath weed population to the point where it is no longer a threat.

One-crop agriculture upsets the balance of nature. Chemical insecticides, fungicides, and herbicides often upset it still further. Biological control measures may be able to replace chemical controls with far less disturbance to the over-all ecology of the region. However, it is crucially important that before such measures are taken, the complete ecology of the introduced organism be studied. Once a natural enemy has wiped out the intended prey, we do not want it to turn to other sources of food and become a nuisance itself. This has happened on occasion. When sugar cane was introduced into the West Indies as a major crop, rats began feeding on it, and the resulting increase in the rat population caused substantial

crop losses. After several unsuccessful attempts at chemical and biological control, the mongoose of India was introduced in the hope that it would rid the islands of rats. It did this quite effectively but, when the job was done, then turned to native wildlife (including valuable predators of other pests) and even domestic animals. The mongoose now ranks as the most troublesome pest of all.

Biological control through the release of sterile males

One of the most ingenious biological control methods yet attempted has resulted in the elimination of the screwworm fly from our southern states. This fly is a serious pest of livestock. The females lay their eggs in sores or other open wounds on the animals, and then the larvae eat the tissues of the host. As they do so, they expose a still larger area to egg-laying, and death of the host is the all-too-frequent outcome.

The eradication of this pest was actually accomplished by releasing some two billion *additional* flies into the natural population. But, before their release, these flies were exposed to just enough gamma radiation to make them sterile but not enough to injure them in any other way.

The success of the undertaking depended only on the sterile males. As their number was increased in an area, the chances of a fertile female in the natural population mating with one increased, too. Each time this occurred, the female would lay sterile eggs. Since the female mates only once, her reproductory career was thus ended without any offspring being produced. After 18 months of this, the pest was totally eliminated east of the Mississippi River. It has since been brought under partial control in the southwestern states as well.

Not only has the technique of swamping a pest population with sterile but otherwise healthy males been shown to be effective, but it has other advantages. In contrast to insecticides, it does not endanger any other animal population unless, of course, the sterile pests should be harmful. The development of resistant strains is not a problem. As the population of the pest grows smaller, the effectiveness of the method increases. Insecticides, in contrast, always miss some individuals, which can then rebuild the population.

The possibility of using sterile males to reduce the population of other pests is now being explored. The direct application of sterilizing chemicals to the natural population seems promising. It would eliminate the need to rear hordes of individuals in captivity but does raise once again the problem of endangering other species of animals.

Another approach that avoids this problem is to exploit the sexual incompatibility that sometimes exists between different strains of the same insect species. For example, mosquitoes from one region often cannot breed successfully with members of their species from another region. This incompatibility probably arises from differences in the cytoplasm of their eggs. In any case, the females who mate with "foreign" males lay infertile eggs just as the screwworm fly females did. Only two months after thousands of male mosquitoes from California were released in an isolated village in Burma, the species there was wiped out. Because the native mosquitoes were carriers of the filarial worm that so often causes elephan-

tiasis (see Section 13.17), an important public health victory was thus achieved.

Exploiting pheromones in insect control

The discovery and synthesis of **pheromones** suggests still another way of killing insects more selectively than insecticide sprays can. Disparlure, the powerful sex attractant of the gypsy moth, may well provide a mechanism by which the male moths in an area could be selectively drawn into poisoned traps. Without mates, the females would be unable to reproduce. Alternatively, the release of large amounts of sex attractant in an area might mask the presence of the females and thus prevent mating. The sex attractants of a number of harmful insects have been isolated or synthesized, and tests indicate that these substances may well provide effective—and selective—control measures against these species.

Man's bypassing of geographical barriers to the migration of plants, animals, and microorganisms has repeatedly upset the balance of nature

The shrinkage of our world, which began with the explorations of the fifteenth century and has yet to cease, has intensified the problem of maintaining a balance of nature. Geographical barriers to the spread of plants and animals are now easily overcome. Columbus probably brought smallpox and measles to the New World. He took syphilis as well as corn (maize) back. The forced migration of Negro slaves from Africa to the West Indian sugar plantations probably introduced malaria and yellow fever to this hemisphere. The transport of crop plants from one part of the world to another has often resulted in a simultaneous transport of their parasites and predators. All too often, the natural enemies of the latter were left at home or failed to become established in the new location.

Transport of raw materials and industrial products from one part of the world to another has also caused the transfer of pests from areas where they were held in natural check to areas where they were not. Elm wood, imported for cabinet making, brought the fungus causing Dutch elm disease and its beetle vectors to our shores in 1930. Since that time, our stately American elm has been dying in increasing numbers. Only vigilant removal of diseased trees and spraying of the insect vector (the elm bark beetle) has enabled many trees to survive. These measures only stem the tide of this fatal disease, however. Unless better control measures are discovered, the American elm may soon vanish as the American chestnut did.

A discussion of the temperate deciduous climax forest written in the early years of this century would have included the American chestnut along with the maple and beech (see Section 10.7); not so today. In 1904, a fungus causing a fatal illness in this species was introduced into this country and virtually wiped out all our chestnuts in a remarkably short period. Today only suckers from the old stumps can be found, and these become infected and die after a few years' growth.

Deliberate transferal of species from one part of the world to another has also frequently led to disaster. The example of the mongoose was mentioned earlier. The gypsy moth, which has caused such damage to trees in the northeastern states, was introduced into Massachusetts in 1869 in hopes that its larvae might be used to start a silk industry in this country. The European rabbit was introduced into Australia for sport in 1859. It



Fig. 33.21 Rabbits in Australia. Having removed all the forage plants, which ordinarily supply them with water as well as food, the rabbits must drink water from a pool. (Courtesy of Dunston, from *Black Star.*)

multiplied explosively in a land where it had no important predators (Fig. 33.21). The raising of sheep (another imported species) suffered severely as the rabbits competed with the sheep for forage. The situation became steadily worse until 1950, when rabbits infected with the myxoma virus were brought from Brazil and released. The epidemic which followed was fantastic. Millions of rabbits (perhaps as many as 99.5% of the population) died. Green grass returned. Sheep raising became easy once again. As extensive preliminary testing with the native marsupial population had indicated, only the European rabbit seems to be susceptible to the virus. Unfortunately, but predictably, resistant rabbits are now appearing.

The human population on the earth today could not be maintained if we did not deliberately upset the natural balance of other populations in our favor. Agriculture, industry, commerce, and speedy transportation have brought changes in our biotic environment which have been beneficial. They have also encouraged secondary changes in plant and animal populations (weeds, rabbits, predacious insects, etc.) which threaten us continually with a sudden reduction in the carrying capacity of the environment. Furthermore, as our influence has spread to every corner of the earth, we have sometimes threatened the very existence of species (e.g. the American chestnut, the blue whale) whose continued presence on earth, while perhaps not indispensable to our welfare, provides interest and variety to our lives.

33.12 WHAT DOES THE FUTURE HOLD?

Renewable vs. nonrenewable resources

With a fast-growing population in a fast-shrinking world, the margin for error in man's handling of his environment has been sharply reduced. If we are going to avoid future catastrophe, we must bring the *long-term* capacity of our environment into balance with our population. We must learn to conserve resources that cannot be renewed (e.g. metal ores, oil, and coal) at least until satisfactory substitutes can be found for them. We must direct our efforts, as much as possible, toward the use of renewable resources for this purpose, that is, materials which are the products of photosynthesis.

Our ever-increasing dependence on renewable resources requires that we do everything we can to avoid reducing the efficiency with which the energy of the sun can be converted into these materials. Soil must be conserved and its fertility carefully maintained. Water must be conserved and its purity guarded. We should also continue to breed plants and animals capable of transforming matter and energy with minimum losses, that is, capable of growing with maximum efficiency. We must exercise the greatest care in transporting species from one region of the earth to another and in applying chemical and biological controls to other populations. All these things will require biologists, in laboratories and in the field, who can unravel the complex ecological interactions which follow any change in the physical and/or biotic environment. It will also require men of affairs with the wisdom and foresight to put into practice the knowledge gained from ecology. Man must, and will, use his environment. He should not use it up. An ecological budget which is unbalanced for too long endangers the physical and spiritual well-being, in fact the very existence, of our future generations. It also endangers the future of the many other living things which share the planet with us and which have taught us so much about ourselves and about the nature of life itself.

EXERCISES AND PROBLEMS

1. List, in the order in which they are brought into play, the natural defenses of the body against bacteria introduced into the dermis by a splinter.
2. Contrast tetanus toxoid and tetanus antitoxin with respect to (a) method of manufacture, (b) mechanism of action in the body, (c) appropriate medical use.
3. With respect to the pig tapeworm why do we say that we are the final host and the pig is the intermediate host rather than the other way around?
4. What are the three problems that must be solved by all internal parasites? How have these been solved by the pig tapeworm?
5. How exactly do populations of DDT-resistant flies arise?
6. What factors regulate the rate of growth of a population of animals started by a single breeding pair introduced into a new territory?
7. What factors limit the ultimate size of this population of animals?

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11. Peakall, D. B., "Pesticides and the Reproduction of Birds," *Scientific American,* Offprint No. 1174, April, 1970.
12. Jacobson, M., and M. Beroza, "Insect Attractants," *Scientific American,* Offprint No. 189, August, 1964. How sex attractants are being used as a weapon against certain insects.
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34 THE ORIGIN AND EARLY EVOLUTION OF LIFE

Theories of the Origin of Life

The Age of the Earth

The Dawn of Life

The Prokaryotes

The Evolution of Eukaryotes

The Photosynthetic Eukaryotic Protists

The Colorless Eukaryotic Protists

The Fungi

The theory of evolution explains the diversity of life on earth today by the descent of each and every species from common ancestors. Certainly the fossil record tells us that in former times, there were fewer species on the earth and that these were not so complex as most of our modern species. In theory, then, the study of evolution should lead us back to a first form of life from which all others have been descended. What was this first form? We do not know, and the best we can do is to make intelligent guesses about its characteristics. Where did it come from? We do not know the answer to this question either, although every thoughtful person has probably wondered about it at one time or another. Even people who know nothing about or disbelieve in the theory of evolution ask themselves how life first appeared on the earth.

What was the first form of life?

34.1 THEORIES OF THE ORIGIN OF LIFE

Among the first attempts at answering this question in our civilization were the stories of creation that are found in the Bible. Other cultures, too, have their stories of the creation of life. These stories share two features in common. First, they were created long before man had gained any knowledge of the physical, chemical, and biological principles that are the basis of life. Second, they invoke divine intervention in the creation of life and thus fall outside the scope of scientific inquiry. Scientists assume that the forces governing the world can be known, that these forces act uniformly at all times and in all places, and that their effects can be predicted, at least in a statistical way. While the scientist's faith may be ill-founded, it is the only basis upon which he can work. If his experiments are subject to unique, supernatural, or capricious intervention, there is no point in performing them. In fact, if some *unrepeatable* result is detected, the scientist assumes that an error in his equipment and/or observation has crept in.

The Cosmozoa Theory. This theory of the origin of life does fall within the scope of scientific inquiry. It explains the presence of life on our earth by assuming it was brought here from elsewhere in the universe, perhaps incorporated in a falling meterorite. Some meteorites do contain organic molecules (e.g. amino acids). While contamination after the meteorite reached the earth can explain the presence of some of these organic molecules, others are not found in living matter here and are thus most likely of extraterrestrial origin. But the arrival of organic molecules from outer space is not the same as the arrival of life. And even if life could withstand the rigors of interplanetary space and the fiery trip through the atmosphere of the earth, the cosmozoa theory really does not answer our basic question. It simply removes it from this planet to some other location.

Extraterrestrial organic matter has been found in meteorites

The Theory of Spontaneous Generation. Until about one hundred years ago, it was commonly believed that life could arise spontaneously from nonliving matter. Van Helmont even gave a recipe—grains of wheat and a dirty shirt in a dark container—for producing mice spontaneously! As

The creation of life from nonliving matter

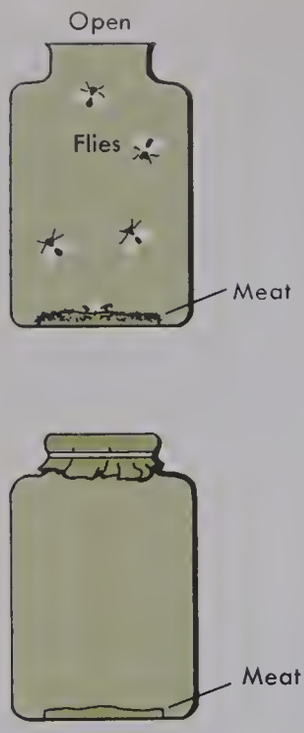


Fig. 34.1 Redi's experiment. Maggots (larval flies) appeared in the meat in the open jar but not in the covered jar.

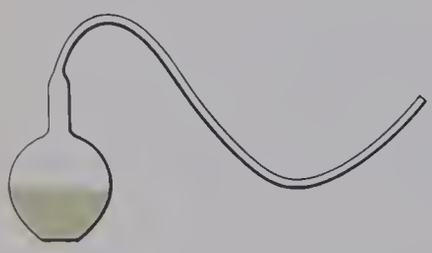


Fig. 34.2 Pasteur's flask. The swan neck permitted fresh air to re-enter the flask after boiling but trapped airborne bacteria before they reached the broth.

more was learned about biology, however, men began to doubt the possibility of spontaneous generation. In 1668 the Italian physician Francesco Redi performed an experiment to show that maggots do not arise spontaneously in decaying meat but are produced from the eggs of flies (Fig. 34.1). Although the spontaneous generation of large forms of life began to be doubted after this, van Leeuwenhoek's discovery of microorganisms reopened the question. Surely these tiny creatures, which appeared so suddenly in rotting food, etc., arose spontaneously! The Italian priest Lazzaro Spallanzani tried to show that even they did not do so. He boiled nutritious broth in glass flasks and then sealed them so that nothing could get into the broth from the outside. The broth remained clear and sterile. Skeptics argued, however, that heating the air within the flask had so altered it that spontaneous generation could not occur.

It was the immortal French biochemist and microbiologist Louis Pasteur who one hundred years ago finally silenced the skeptics. He, too, boiled flasks of broth, but instead of sealing the necks of the flasks, he drew them out into an *S*-shape, leaving the ends open (Fig. 34.2). Now fresh air could reach the interior of the flask but, Pasteur reasoned, any bacteria or other microorganisms floating in it would be trapped in the long neck. Sure enough, the broth usually stayed sterile until he tipped a little into the neck of the flask and then allowed it to run back. Only after doing this, did microorganisms begin to grow in it. Some of the flasks that Pasteur prepared are still in existence (now sealed), their contents of broth clear and pure.

Two important points about Pasteur's work are often overlooked. First of all, he did not prove that spontaneous generation can never and has never occurred. It is virtually impossible to prove the negative of something. Pasteur, like Redi before him, simply showed that supposed examples of spontaneous generation failed to work if already living organisms were kept away. Actually, we have good reasons for thinking that life is not created spontaneously today but we certainly have no rigid proof of this.

The second point about Pasteur's work is that if it does really mean that the spontaneous generation of life is impossible, then the problem of the origin of life cannot be explained on scientific grounds. Fortunately, a way out of this dilemma was proposed in 1936 by the Russian biochemist A. I. Oparin.

The "primeval soup"

Oparin's Theory. While conceding that life does not arise spontaneously now, Oparin felt that it might well have arisen spontaneously under the conditions that existed earlier in the history of the earth. According to this theory, the oceans of the early earth contained a rich supply of organic molecules. (They do not today. Can you guess why not?) Over a vast span of time, these molecules became associated with one another in temporary complexes. Ultimately, one such complex developed: (1) some sort of membrane to separate it from the "soup" of organic molecules around it, (2) the ability to take in molecules from this soup and discharge other

molecules into it, (3) the ability to incorporate the absorbed molecules into the characteristic pattern of the complex, and (4) the ability to split apart portions of itself that had all these features, too. Such a complex could have been the first living thing. Metabolism, growth, and reproduction, perhaps even responsiveness, would have been present and hence all the attributes of life. From this first form, surely far simpler than even the simplest microorganisms alive today, could have evolved by natural selection the present diversity of life.

Atmosphere of the early earth

The plausibility of Oparin's theory depends greatly on the earth's being somewhat different then than it is now. In order for organic molecules characteristic of life to have accumulated in the oceans, there must have been substantial concentrations of the inorganic ingredients out of which they could be synthesized and a source of energy to accomplish these syntheses. Analysis of the atmosphere of the large, cold planets, Jupiter and Saturn, reveals the presence of methane (CH_4) and ammonia (NH_3). If these two gases, together with water vapor (H_2O) were present in the atmosphere of the early earth, all the elements would have been present (C, H, O, N) for the synthesis of most of the amino acids, the carbohydrates and fats, and the purines and pyrimidines. Radioactivity, ultraviolet radiation from the sun, heat from volcanos, and electrical discharge all might have provided energy for the synthesis of various organic molecules from these gases.

Miller first showed that organic molecules could be synthesized under conditions thought to exist on the early earth

Could such a system really work? A student of biochemistry, Stanley Miller, sought to answer this question as part of his work toward a Ph.D. degree. He built the apparatus illustrated in Fig. 34.3 and filled it with water, methane, ammonia, and hydrogen. This mixture was kept circulating by continuously boiling and then condensing the water. The gases passed through a chamber containing two electrodes with a spark passing

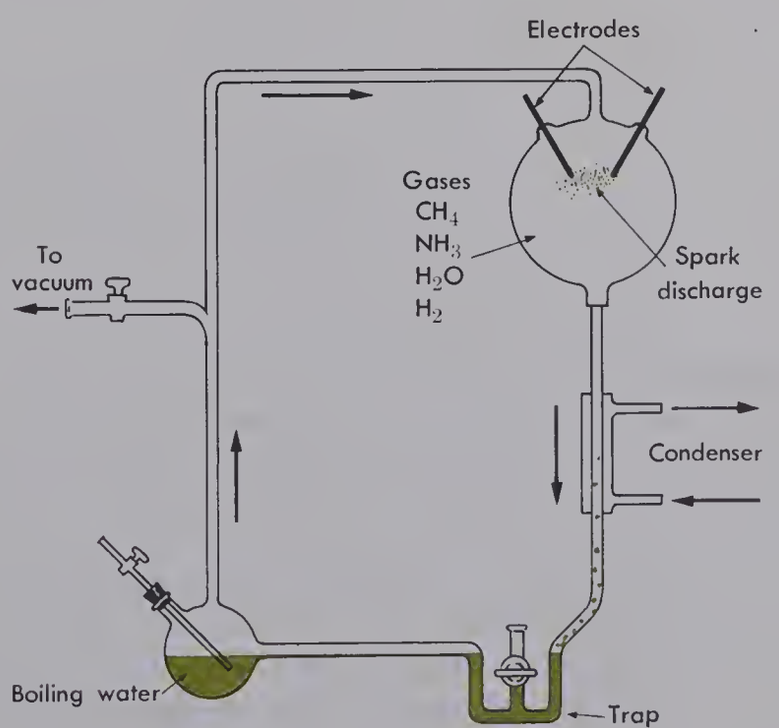


Fig. 34.3 Miller's apparatus. After running for one week, a variety of organic molecules had formed in it.

continuously between them. At the end of a week, chemical analysis of the contents of the flask revealed the presence of several amino acids (e.g. glycine, alanine) and other organic molecules including one, succinic acid, which is found in the citric acid cycle. Miller's results encouraged others to vary his procedure, using different mixtures of substances that might have been present on the primitive earth and other sources of energy. Most of the other amino acids, some small polypeptides, purines and pyrimidines, sugars, and a vitamin (nicotinamide) have been produced in these later experiments.

None of these experimental re-creations of what might once have been the conditions on the earth has produced anything alive. Undeniably, though, they have produced some of the molecules uniquely associated with life. Could these have eventually associated themselves into a complex with the attributes of life? We may never know. Such an event would be extremely unlikely and a week, or even a million years, might not be long enough for the necessary chance association to occur. Furthermore, there must have been some way in which organic molecules, once formed, could be protected from degradation by the same forces that created them. In Miller's apparatus, the electric spark can break organic molecules back down as easily as it promotes their synthesis. Dissolved in the oceans of the primitive earth or adhering to the surface of minerals, complex associations of organic molecules might have been better protected against breakdown.

In any case, one sure requirement for an event such as the spontaneous creation of life must have been a vast span of time. On uncountable occasions, complexes must have almost reached the point where they would have been alive only to become degraded again. Was there enough time for chance to finally succeed?

34.2 THE AGE OF THE EARTH

Not until little more than a century ago did men begin to appreciate just how old the earth is. Without a knowledge of the facts of geology and geochemistry, they simply could not conceive of the great length of the history of the earth. One biblical scholar, Archbishop Ussher, calculated from his studies that the earth was created at 9:00 A.M. on October 12 in the year 4004 B.C. However, calculations based on geological studies produced quite a different picture of the antiquity of the earth. One of the first estimates was based on the increasing salinity of the oceans. Dividing the salt concentration of our present oceans by their annual increase in salinity, one arrives at a figure of about 50 million years for their age. Unfortunately, this method requires us to assume that the oceans have been getting saltier at a constant rate through the years, and we have no grounds for believing this. A similar attempt was made to establish the age of the earth by measuring the total thickness of sedimentary rock deposits and dividing this by the estimated annual increase in the thickness of ocean sediments. Although this method doubled previous estimates, it suffered from the

Methods for estimating the age of the earth

same defect as the first. Perhaps sediments were deposited more or less rapidly at other periods in the history of the earth than they are today. An additional problem was the fact that a continuous record of sedimentary rock is not available, for erosion has erased entire chapters of this portion of the geological history of the earth.

Certain radioactive isotopes provide clocks for measuring the ages of rocks and fossils

Is there a geological process which we can be sure proceeds at a constant rate? The discovery of the radioactive elements has provided us with one. Radioactive elements contain unstable isotopes which, by emitting subatomic particles such as electrons and alpha particles (helium nuclei), become "transmuted" into other isotopes. Atoms of U-238, the most abundant isotope of uranium, undergo a series of transmutations as they "decay" radioactively. Each atom in the series is also unstable and itself decays. Finally, though, an isotope of lead (Pb-206) is formed that is stable, and the process stops. The important thing about these transformations from our point of view is that they take place at a definite, measureable rate. It takes 4.51 billion years for half the atoms in any sample of U-238 to decay. (We call this period the "half-life" of the isotope.) Neither high temperatures nor low, neither gravity, magnetism, electrical currents nor any other physical or chemical force can alter this inexorable rate. Here,

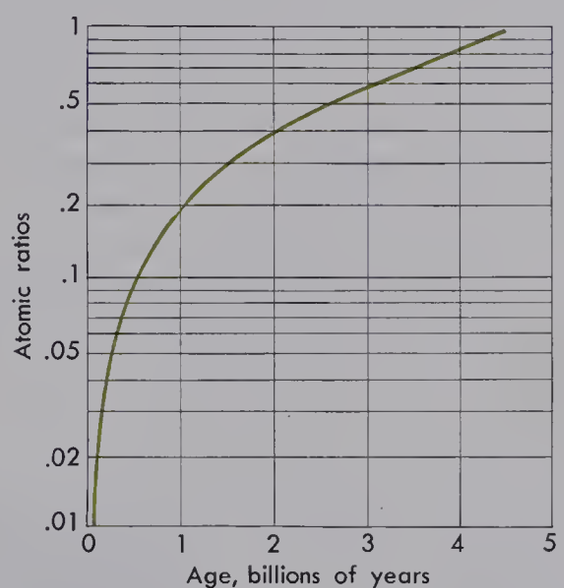


Fig. 34.4 Change in ratio of Pb-206: U-238 over a period of 4.5 billion years.

then, is a geological clock which keeps perfect time and whose elapsed running time can be computed simply from the ratio of Pb-206 atoms to U-238 atoms in a rock sample (Fig. 34.4). Fortunately, delicate chemical and physical methods exist for making these determinations. The oldest rocks to be dated by this method are from Southern Rhodesia. They are 2.7 billion years old. However, they surely were not formed at the time the earth itself was formed. The study of other isotopes in other minerals (as well as in meteorites) indicates that the earth (and the solar system) was formed some 4.7 billion years ago.

34.3 THE DAWN OF LIFE

No one knows exactly when life first appeared on the earth. We would not expect the first forms of life to leave fossil remains for us. However, fossils that resemble bacteria have been found in rocks over three billion years old (Fig. 34.5). This still leaves us with almost two billion years for life to have arisen on the earth. Even the most unlikely event will probably occur sometime. Two billion years provides plenty of time for false starts in the chance production of the first living organism.

The earliest life could have secured its energy by fermenting the organic molecules around it

Nourishment for the first form of life was no problem. Surrounded by the same soup of organic molecules from which it arose, it had only to use these molecules to supply its needs for energy and the materials for growth and reproduction. We assume that the first living organism secured its energy from these organic molecules by fermenting them. As we shall see below, life probably could not have arisen if oxygen had been present in the primitive atmosphere, and for several reasons we believe that there was none.

Fermentation could not continue indefinitely as the only way of life of the earliest organisms. No matter how large the primeval soup of molecules, eventually it would have become exhausted. If this experiment in life was not to cease then, some organisms must have evolved a means of synthesizing new organic molecules from the inorganic substances found in the environment. Such synthesis requires energy and probably the energy came from the sun. The appearance of photosynthetic organisms not only provided an inexhaustible supply of organic molecules to meet the needs of life but ultimately provided oxygen as well. With the accumulation of oxygen in the atmosphere, the way was open for heterotrophic organisms to secure their energy needs by the vastly more efficient process of cellular respiration. With an inexhaustible supply of energy-rich molecules and an efficient method for extracting energy from them, the full potentialities of life could be realized.

The evolution of autotrophy was a prerequisite for the continuation of life

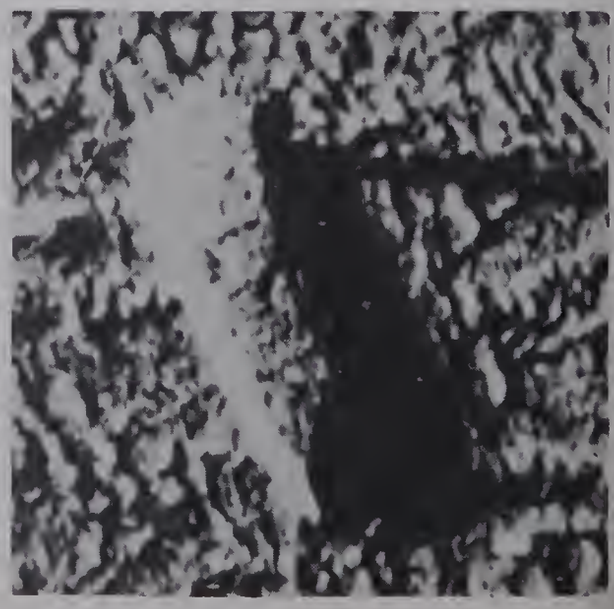


Fig. 34.5 Fossil bacterium. This fossil was found in South Africa in sedimentary rock over three billion years old. It is the oldest evidence of life yet discovered. This prokaryote is about 0.5 μm long and has been named *Eobacterium isolatum.* (Electron micrograph courtesy of E. S. Barghoorn from E. S. Barghoorn and J. W. Schopf, *Science,* 152, 758–763, May 6, 1966.)

Does life arise spontaneously today?

If our theory of the spontaneous generation of life is correct, does it mean that Redi, Spallanzani, and Pasteur were wrong? Not at all. The theory Redi and the others tried to refute was that modern forms of life arise spontaneously. On the other hand, Oparin's theory is that an organism, far more primitive and simple than any that lives today, arose once in the history of the earth. (Our conviction that this event occurred just once is based on the remarkable similarities of biochemistry and subcellular structure in all living things.) We believe that additional spontaneous generations could not occur today. For one thing, there are no longer appreciable quantities of organic molecules in the waters of the earth. Second, any complex of organic molecules that might form spontaneously would quickly be destroyed by (1) the oxygen which is now present in the atmosphere and acts as a powerful oxidizing agent and (2) the already existing forms of life (for example, bacteria) which would consume our hopeful complex long before it developed to the point where we could consider it to be alive.

34.4 THE PROKARYOTES

If one examines the anatomy of the cells of the diverse creatures that populate the earth, a basic dichotomy becomes apparent. The cells of the majority of organisms contain nuclei, mitochondria, plastids (if they are photosynthetic), and most of the other structures that we examined in Chapter 5. Such cells are called eukaryotic ("truly nuclear") and their owners are the **eukaryotes.** However, the cells of bacteria (and their relatives) and the cells of blue-green algae do *not* have these organelles. These creatures are called **prokaryotes** ("pre-nuclear"). Prokaryotes differ from eukaryotes in a number of other ways. Their single chromosome is not associated with histones, they show no evidence of cytoplasmic streaming, their ribosomes are constructed differently, and (as we shall see later) they share a number of biochemical peculiarities not found in the eukaryotes. The prokaryotes differ in such fundamental ways from the eukaryotes that many biologists choose to classify them in a separate kingdom: Monera.

Prokaryotes vs. eukaryotes

The Monera

While no one should underestimate the functional complexity of prokaryotes (they can pull off a number of metabolic tricks that we cannot), nevertheless we have good reason to consider them the little-changed descendants of the earliest forms of cellular life. In this sense, then, they are *primitive.* Not only does the limited fossil evidence suggest that prokaryotes preceded eukaryotes (Fig. 34.5), but the metabolic abilities of certain prokaryotes surviving today would have suited them to the conditions that we assume existed earlier in the earth's history. The green photosynthetic bacteria, for example, live on the surface of ocean mud and, like the purple sulfur bacteria (see Section 9.8) manufacture organic compounds from CO_2, H_2S, and the energy of sunlight.

Photosynthetic bacteria are not able to use water as a source of electrons

$$CO_2 + 2H_2S \xrightarrow{\text{light}} (CH_2O) + H_2O + 2S$$

Their light-absorbing pigment resembles, but is not identical to, the chlorophyll of plants. In the dark, they secure energy by the fermentation of organic molecules. In fact, these organisms cannot grow in the presence of oxygen. They would therefore have been perfectly able to survive in the atmosphere of the primitive earth.

From such primitive, H_2S-using photosynthetic bacteria could have evolved forms capable of using water as the source of hydrogen with which to reduce carbon dioxide to carbohydrates.

$$CO_2 + 2H_2O \xrightarrow{\text{light}} (CH_2O) + H_2O + O_2$$

The blue-green algae may have begun the building of an oxidizing atmosphere

Photosynthesis by such organisms—the blue-green algae are the most likely candidates—would have liberated oxygen into the atmosphere and thus established the conditions needed for cellular respiration and the greatly increased efficiency of energy utilization that it makes possible.

The Bacteria. Bacteria are found literally everywhere. Soil teems with them. Their spores have been recovered from air samples collected at an altitude of 45,000 feet. Bacteria are found on the surfaces (both external and internal) of plants and animals. Many live commensalistically in such locations but may, when the conditions are right, become frankly parasitic. Others are invariably damaging to any host they enter. Figure 34.6 lists some human diseases caused by the three major morphological types of bacteria.

Some Diseases Caused by Bacteria

1. Coccus forms:
   - rheumatic fever
   - gonorrhea
   - scarlet fever
   - some kinds of pneumonia
   - carbuncles
   - some kinds of meningitis
2. Bacillus forms:
   - diphtheria
   - typhoid fever
   - bubonic plague
   - tetanus
   - tuberculosis
   - anthrax
   - whooping cough
   - botulism (from toxin in spoiled food)
   - leprosy
   - tularemia
   - undulant fever
3. Spirillum forms:
   - asiatic cholera

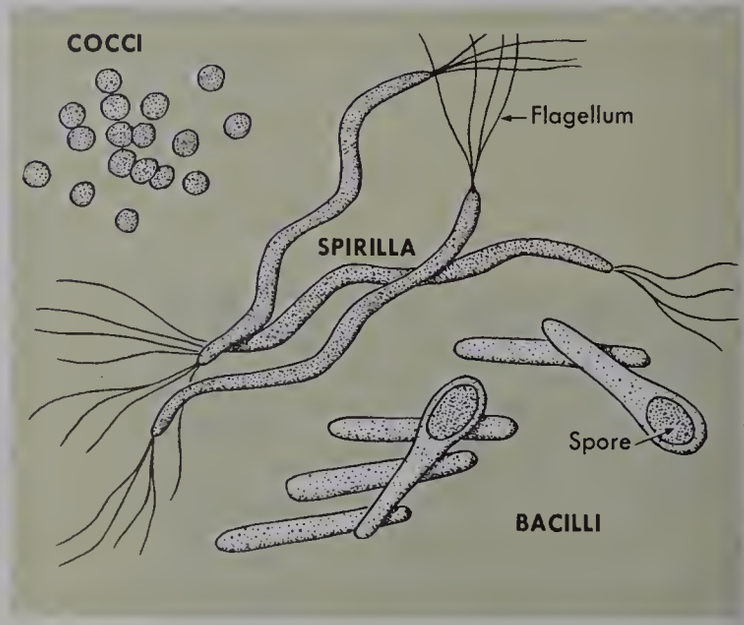


Fig. 34.6 Types of bacteria and some diseases caused by each.

Most bacteria are beneficial

We have seen numerous examples in this book of beneficial roles that bacteria play. They are essential not only for the cycling of nitrogen in the biosphere (Fig. 11.3), but for the cycling of all the other nutrients. Man exploits the talents of bacteria in such activities as converting alcohol into vinegar, souring milk for the production of cheese, and breaking down sewage into inoffensive products.

Spirochetes

Three other groups of microorganisms are often included with the bacteria because of their similarities in cell structure, size, and ecology (they all include species that cause disease). The spirochetes are long, thin, spiral-shaped microorganisms that range in length from just a few to as many as 500 μm (Fig. 34.7). Unlike the "true" bacteria, they do not have a rigid cell wall and thus are free to bend easily. Although some spirochetes live harmlessly in fresh water, soil, or in the bodies of animals, others are serious parasites. Perhaps the most notorious of these is the spirochete that causes the human veneral disease, syphilis.

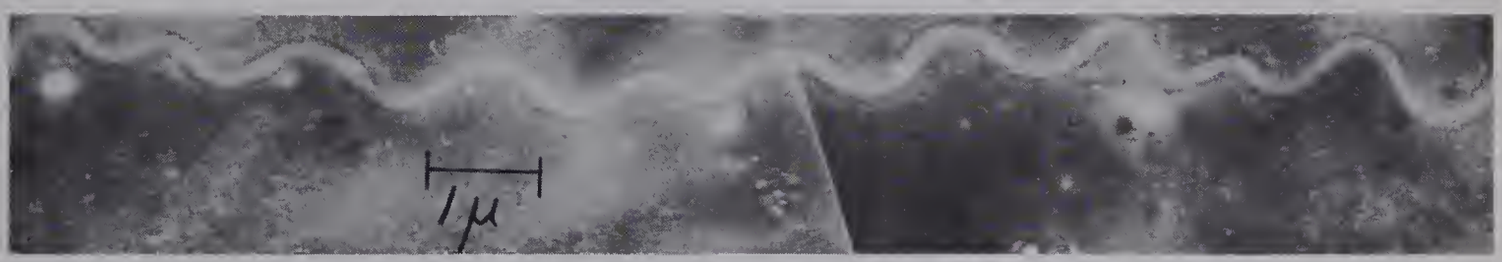


Fig. 34.7 The spirochete that causes syphilis. (Courtesy of Harry E. Morton.)

Mycoplasmas

In recent years an increasing amount of attention has been given to a small group of microorganisms called mycoplasmas. In many ways they resemble tiny bacteria without cell walls. Some are free-living while others are parasites. One of the latter causes a type of pneumonia, called pleuropneumonia, in man. For this reason, the mycoplasmas are also known as the pleuropneumonia-like organisms or PPLO.

The mycoplasmas have the distinction of including the smallest free-living organisms known. Although many are so small (0.1 μm) as to be visible only under the electron microscope, they contain everything needed to carry on all the fundamental activities of life.

Rickettsias

Rickettsias, like the mycoplasmas, are so small that they can barely be distinguished in the light microscope. They differ from the mycoplasmas in that all of them are obligate parasites. This means that they can grow and reproduce only so long as they are present within the living cells of a host organism. In some cases they live harmlessly in the cells of their host; in other cases they cause symptoms of disease. Typhus fever is caused by a rickettsia injected into man by the bite of the body louse. Rocky Mountain spotted fever is caused by a rickettsia transmitted to man through the bite of a tick.

The Blue-Green Algae. The blue-green algae get their name from the blue pigment that, along with chlorophyll, is always present in their cells. Some species have a red pigment as well, and this may cause them to appear red or purple and not blue-green at all.

Fresh water, salt water, and soil are all inhabited by blue-green algae. These organisms are exceedingly hardy. The black band found at the high-tide mark on rocks along the sea coast is formed by blue-green algae (Fig. 34.8). Some species grow luxuriantly in the hot springs of Yellowstone National Park at temperatures hot enough to cook an egg (161°F).

Some blue-green algae can fix atmospheric nitrogen

A number of blue-green algae can fix atmospheric nitrogen. It is these species that "bloom" when phosphates (but not nitrates) become available in lakes and other bodies of fresh water. Nitrogen-fixing blue-green algae are also important in maintaining the fertility of rice paddies.

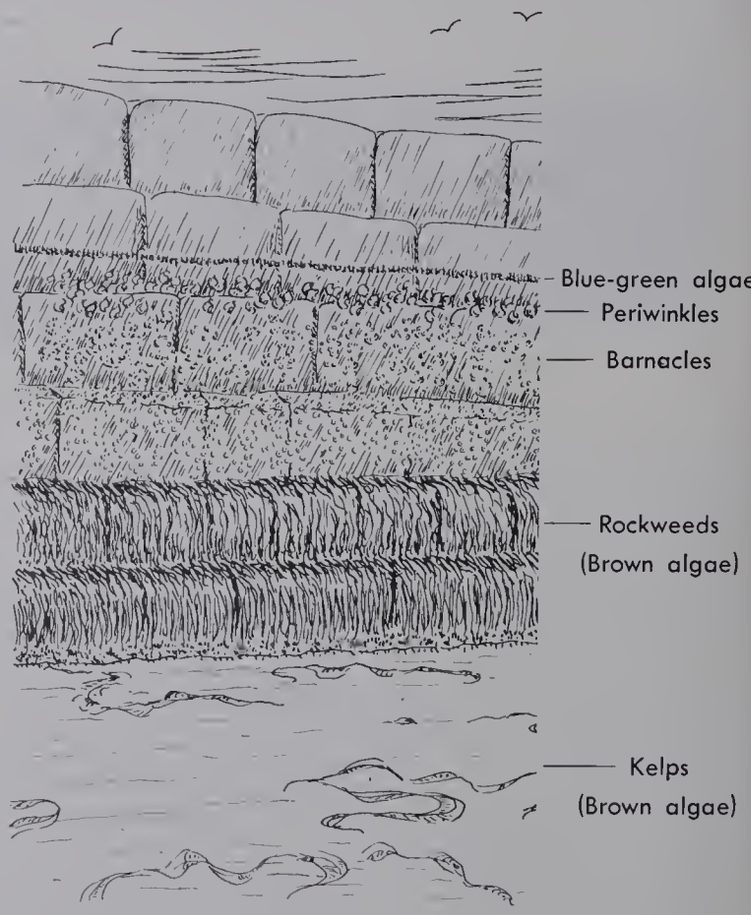


Fig. 34.8 Low tide reveals the characteristic zonation of sessile algae on a breakwater. Each zone also has its characteristic animals. Only organisms adapted to withstand violent wave action and periodic exposure to the air can survive here. Despite the harsh conditions, such a habitat is teeming with life.

34.5 THE EVOLUTION OF EUKARYOTES

By any criterion, the eukaryotes dominate the earth. Of the 1.2 to 2 million kinds of living organisms found on the earth today, all but a few thousand are eukaryotes. How should this enormous assemblage be classified? Some biologists would prefer two kingdoms: the plants and the animals. However, such a scheme results in some forms (e.g. kelps, yeast) being included with the plants even though they are probably no more closely related to a grass plant than you are. We shall, then, divide the eukaryotes into four

The four major groups of eukaryotes

major groups (call them kingdoms if you wish): the protists, the fungi, the plants, and the animals. Of these, the eukaryotic protists were probably the first to evolve. Whence did they arrive?

The endosymbiont theory of the origin of eukaryotes

Perhaps the most intriguing hypothesis that attempts to answer this question is that the eukaryotic protists evolved by the *symbiotic association* of two or more kinds of prokaryotes. According to this theory, the mitochondria of eukaryotic cells were once aerobic bacteria living symbiotically within their host. Chloroplasts are thought to be derived from prokaryotic algae (e.g. the blue-green algae). It has even been suggested that endosymbiotic spirochetes provided the first flagella and cilia, and, with these, the basal bodies from which the spindle could be constructed and mitosis made possible. Let us examine some of the evidence upon which this theory is based.

Mitochondria and chloroplasts have genetic machinery resembling that of prokaryotes

Both mitochondria and chloroplasts (also basal bodies) are *semi*-autonomous in the sense that they can duplicate themselves independently of the duplication of the cell in which they reside. This property becomes more remarkable when we realize that both mitochondria and chloroplasts have rudimentary genetic systems quite separate from the genetic system in the nucleus. Each has DNA and its own protein-synthesizing machinery. The extraordinary thing from the point of view of our hypothesis is that this genetic machinery is so similar to that of prokaryotes. The DNA is present as a single molecule, just as it is in bacteria and blue-green algae. It is not complexed with histones like the DNA of eukaryotic chromosomes. The relative proportion of G + C to A + T in mitochondrial DNA is like that of bacterial DNA, not eukaryotic DNA. Chloroplast DNA from *Euglena,* a eukaryotic protist, shows extensive sequence homologies with the genes for ribosomal RNA in blue-green algae.

The protein-synthesizing machinery of mitochondria and chloroplasts resembles that of prokaryotes

The parallels extend to the protein-synthesizing machinery. The size and properties of the ribosomes found within mitochondria and chloroplasts are like those of bacteria and blue-green algae, not like those around them in the eukaryotic cytoplasm. A number of antibiotics, e.g. streptomycin, exert their effect by interfering with protein synthesis in bacteria but not in their eukaryotic host. These antibiotics do, however, inhibit protein synthesis within mitochondria and chloroplasts. Conversely, inhibitors of protein synthesis in eukaryotic cytoplasm such as diphtheria toxin have (sensibly enough) no such effect *either* on bacterial protein synthesis *or* on protein synthesis within chloroplasts and mitochondria. When ribosomes from *Euglena* chloroplasts are dissociated into their components and then allowed to reassociate with complementary subunits from *E. coli* ribosomes, the hybrid complexes that result work perfectly. The antibiotic rifampicin, which inhibits the RNA polymerase of bacteria, has no such effect on the RNA polymerase within the eukaryotic nucleus, but does inhibit the RNA polymerase within mitochondria.

Even today, some heterotrophs exploit intracellular autotrophic symbionts

All of this tells us that chloroplasts and mitochondria share many properties with prokaryotic cells. But what of evidence that prokaryotes can indeed form endosymbiotic relationships? We observed in the last chapter that a number of heterotrophic organisms exploit photosynthetic

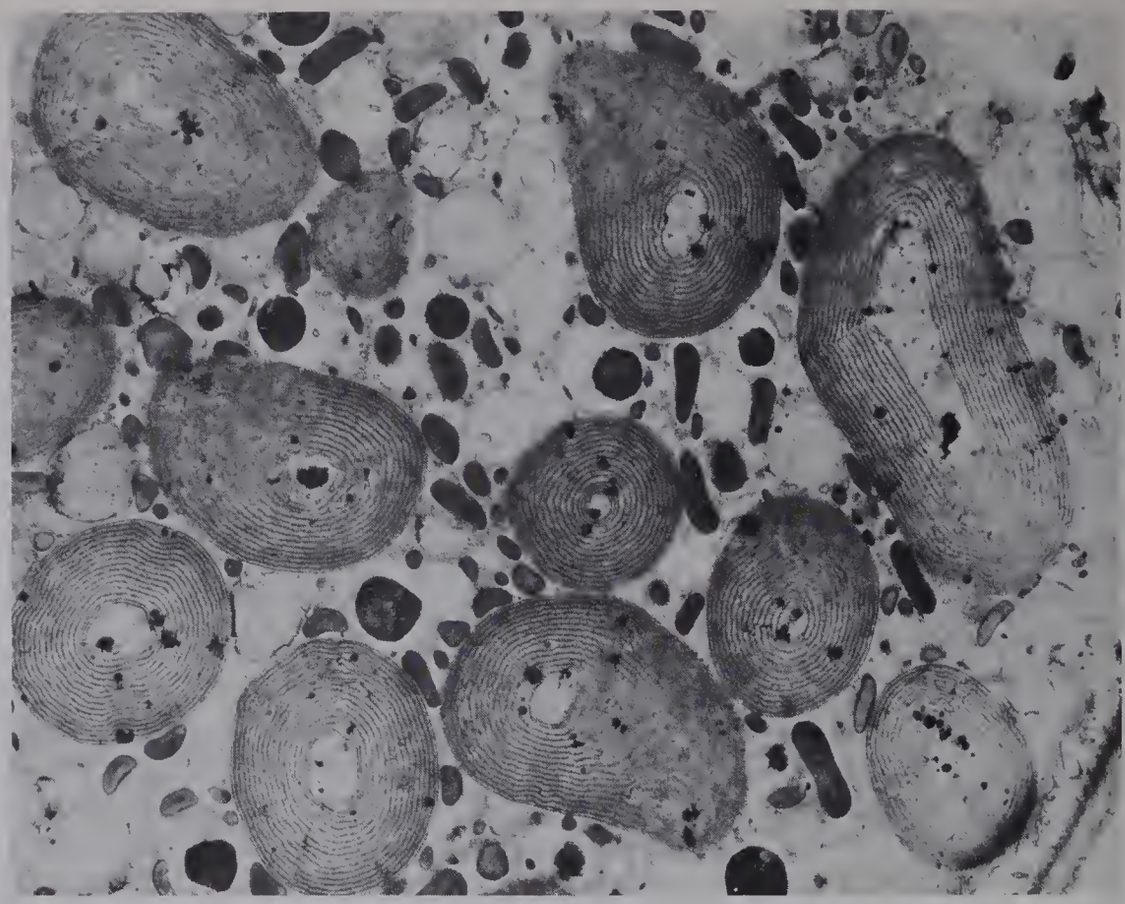


Fig. 34.9 *Glaucocystis nostochinearum,* a unicellular green alga (Chlorophyta). It has no chloroplasts of the type normally found in green algae, but carries on photosynthesis by means of organelles that resemble *blue-green* algae (large ovals). Note portion of the cell wall at the lower right. (10,000 X, courtesy of Patrick Echlin.)

endosymbionts. In many cases, these endosymbionts are prokaryotes, i.e. blue-green algae. Figure 34.9 is an electron micrograph of a unicellular green alga that has lost the **chloroplasts** typical of its group but which is still able to carry on photosynthesis thanks to its acquisition of structures that resemble blue-green algae. The similarities between these "organelles" and blue-green algae occur not only in their general structure (Fig. 34.10, left) but also in a number of their biochemical features. Figure 34.10 also shows (right) the single chloroplast of a eukaryotic **red alga.** Here again, the chloroplast bears striking structural and biochemical similarities to blue-green algae. Note, however, that in both of these cases (*Glaucocystis* and *Porphyridium*), the relationship between the cell and its organelles is now, at least, obligatory. While chloroplasts (and mitochondria) still possess their own genes, they also depend upon nuclear genes of their "host" for essential functions (e.g. synthesis of cytochrome enzymes). However, the previous chapter certainly should have prepared us for the loss of function that is so often the outcome of symbiotic relationships and, of course, invariably of those that are obligatory.

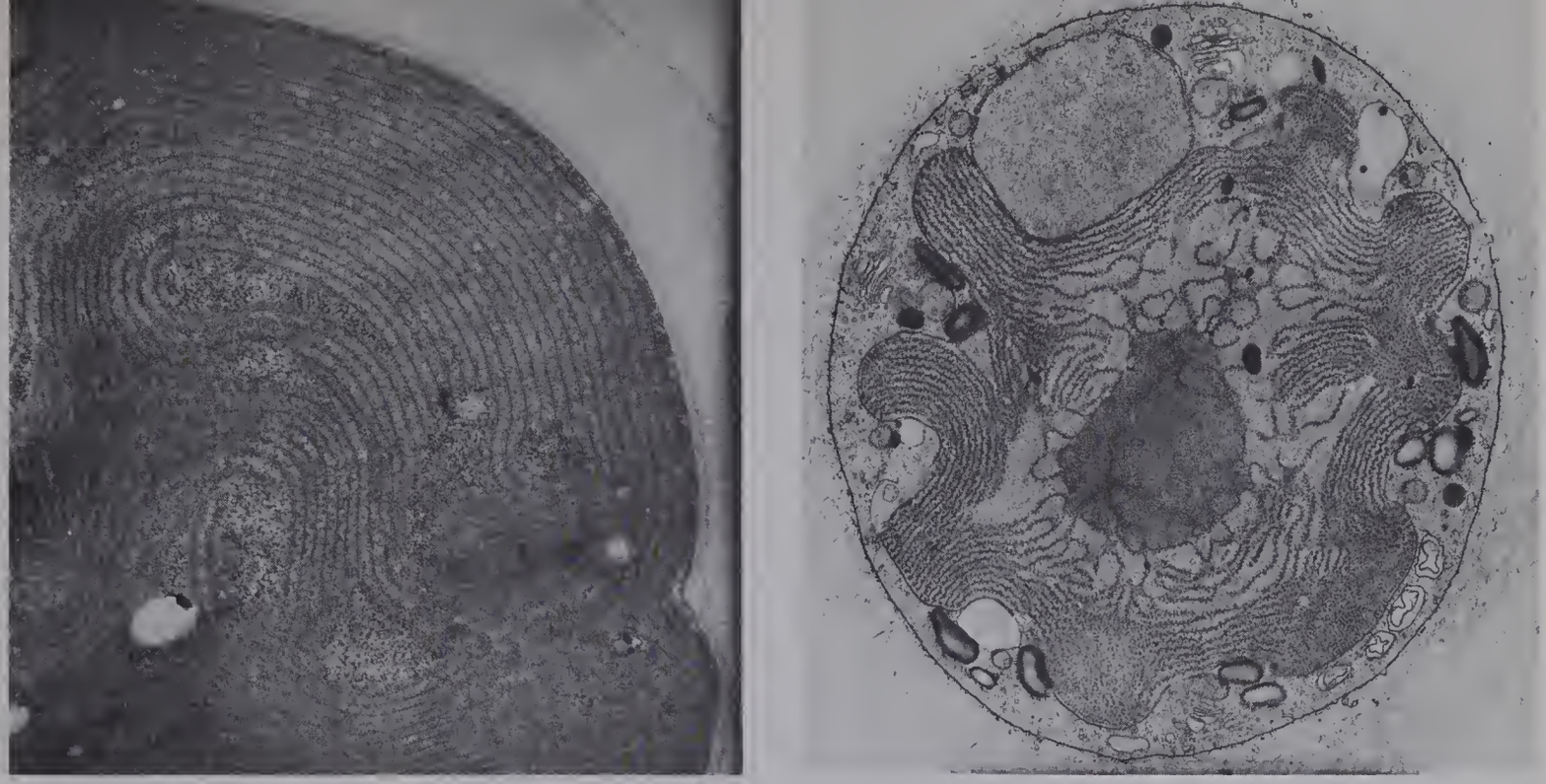


Fig. 34.10 Left: electron micrograph (25,000 X) of a blue-green alga. (Courtesy of Dr. G. Cohen-Bazire.) Right: *Porphyridium,* a unicellular red alga. (Electron micrograph 10,000 X, courtesy of Dr. E. Gantt.) The organization of the membranes in the single chloroplast of the red alga and the pigments they contain are quite unlike the chloroplasts of other photosynthetic eukaryotes, but quite similar to prokaryotic blue-green algae.

34.6 THE PHOTOSYNTHETIC EUKARYOTIC PROTISTS

What were the first organisms that might have appeared as the outcome of an association between two or more kinds of prokaryotes? While we can only speculate, two likely candidates would have been the ancestors of two kinds of algae found today: the **red algae** and the dinoflagellates. We have already noted the similarity between red algae and blue-green algae and the list could be extended. A number of red algae are unicellular, although large multicellular forms are also found. Perhaps the latter evolved by the continued association of daughter cells in colonies in much the same way we postulated for the relatives of *Volvox* (Fig. 5.16).



A dinoflagellate

The **dinoflagellates** are exclusively unicellular. Like the red algae, they exhibit a number of traits (e.g. in cell division) that appear intermediate in nature between those of prokaryotes and those of the more advanced eukaryotes. Unlike the red algae, dinoflagellates have the eukaryotic type of flagellum (with the "9 + 2" arrangement of fibrils—see Fig. 5.11), another major acquisition of life. Aside from their scientific interest, dinoflagellates become of concern to us when from time to time they reproduce explosively and create the poisonous red tides that may cause extensive kills of marine fish and make filter-feeding marine organisms like clams unfit for human consumption.

There are a number of other groups of eukaryotic algae. Four of these are of special interest.

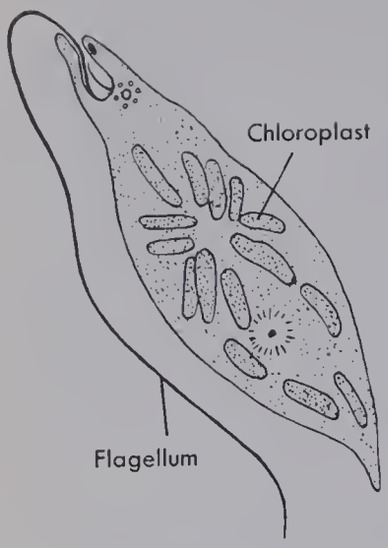


Fig. 34.11 *Euglena,* 1200 X.

1. The Euglenophytes (Euglenophyta). These organisms are photosynthetic flagellates that are not encased in a rigid cell wall. *Euglena* is a typical member of the group. Lacking a cell wall, it can change shape easily. It moves rapidly by means of a long flagellum at the front end (Fig. 34.11). The euglenophytes have both chlorophyll *a* and chlorophyll *b*.

2. The Green Algae (Chlorophyta). These algae resemble the euglenophytes in their photosynthetic pigments (chlorophyll *a* and *b*). However, their cells are encased in a rigid wall made of cellulose. Some of the green algae are flagellated (e.g. *Chlamydomonas, Volvox*), and even those that are not (e.g. *Ulva*—Fig. 34.12) produce flagellated gametes and/or zoospores. The presence of chlorophyll *a* and *b* and of a cellulose cell wall suggests that the green algae are the closest protistan relatives of the plants.

When both phosphates and nitrates are abundant, freshwater green algae often form extensive algal "blooms."

3. The Golden Algae (Chrysophyta). These algae get their name from a yellow-brown pigment that is found (in addition to chlorophyll) within their cells. Most of them are unicellular and many are flagellated, although the most important members of the phylum, the **diatoms,** are not. Diatoms have a cell wall or shell that is made up of two overlapping halves. These shells are impregnated with silica and are often beautifully ornamented. In fact, the delicate sculpturing of the shells of some species provides a good test of the quality of microscope lenses (Fig. 34.13).

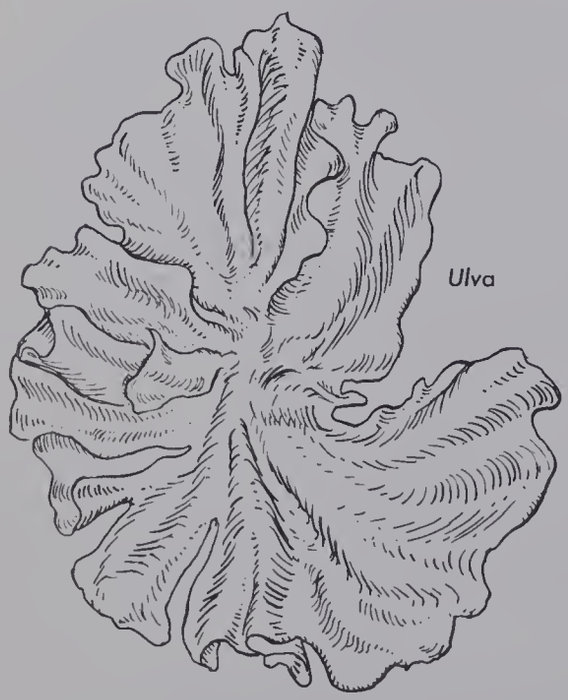


Fig. 34.12 *Ulva* ("sea lettuce"), a green alga that grows in the marine intertidal zone. This organism consists of a double layer of cells and may grow to a diameter of three feet.



Fig. 34.13 A diatom shell. (200 X, courtesy of the General Biological Supply House, Inc.)



Fig. 34.14 Seaweeds exposed at low tide. Two species of brown algae and one red alga (the mossy material at the water line) grow here tightly attached to the rocks.

Diatoms start many aquatic food chains

Diatoms play an important role in the economy of nature. Both in fresh water and in the oceans, they accomplish a major part of all the photosynthesis that occurs. Thus they serve as a vital source of food materials for many colorless protists and small animals. These, in turn, serve as food for still larger animals. As the main food producers in aquatic environments, the diatoms thus sustain a multitude of other, nonphotosynthetic organisms.

The glassy shells of the diatoms do not decay after death. Consequently they may accumulate in deep layers at the bottom of the ocean in regions where diatoms are especially abundant. In some cases, these deposits have, millions of years later, been lifted above sea level by geological forces. This has occurred in California and the deposits are now mined and sold as "diatomaceous earth." This material is used as a filtering agent for clarifying liquids and is also incorporated in soundproofing materials. The countless numbers of tiny glassy shells make a fine abrasive, and scouring powders, silver polish, etc. often contain diatomaceous earth.

4. The Brown Algae. The members of this group have a brown pigment which masks the green of the chlorophyll they also possess. They are all multicellular, somewhat plantlike forms found almost exclusively in salt water. The rockweeds, which form a dense mat on rocks exposed to the changing tides (Fig. 34.14), and the kelps are large and widespread members of the phylum. There is some specilization of parts in these organisms, and many have rather complex life cycles. Giant kelps along our Pacific coast grow as long as 100 feet. Despite their great size, the organization of these forms is still quite simple compared with that of true plants.

Brown algae are used for food in some coastal areas of the world and, in this country, as a source of fertilizer and iodine.

34.7 THE COLORLESS EUKARYOTIC PROTISTS

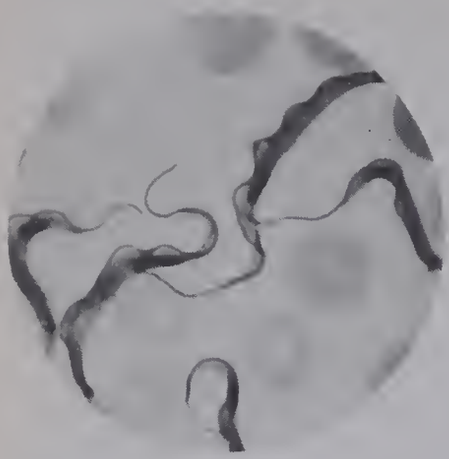


Fig. 34.15 Trypanosomes in human blood, 1100 X. African sleeping sickness is caused by a trypanosome that is transmitted to humans through the bite of the tsetse fly. (Courtesy of CCM: General Biological, Inc.)

The Protozoa. Any unicellular, heterotrophic, motile organism qualifies to be a member of this group. These organisms are further divided according to their mode of locomotion:

a) by pseudopodia (e.g. *Amoeba*),

b) by flagella (e.g. the trypanosome that causes African sleeping sickness —Fig. 34.15),

c) by cilia (e.g. *Paramecium*—Fig. 34.16).

In addition, there are some microorganisms that are obviously protozoans even though they have lost the power of locomotion during part or all of their lives. These, the sporozoans, are exclusively parasitic, and include the organisms that cause malaria, perhaps the most devastating of man's parasitic diseases.

There is nothing primitive or simple about the ciliated protozoans. Forms such as *Paramecium* are not only large for single cells, but have many organelles which parallel in function the organs found in multicellular creatures. Indeed, the complexity of the ciliates has led some biologists to consider them acellular (not cellular) rather than unicellular organisms. What they wish to stress is that the *Paramecium* "body" is far more elaborate in its organization than any cell out of which multicellular organisms are made. Whether this is a sound approach or not, the fact remains that the ciliates are the most elaborate of all the protozoans.

Some protozoans move by both cilia and flagella. They may have both at one time (Fig. 34.17) or develop one or the other as conditions dictate. Furthermore, a number of flagellated protozoans look just like certain motile algae and would be classified with these "twins" if only they had chlorophyll. We must conclude, therefore, that to group all the protozoans on the basis of method of locomotion is justified more by convenience than by genuine evolutionary kinship.

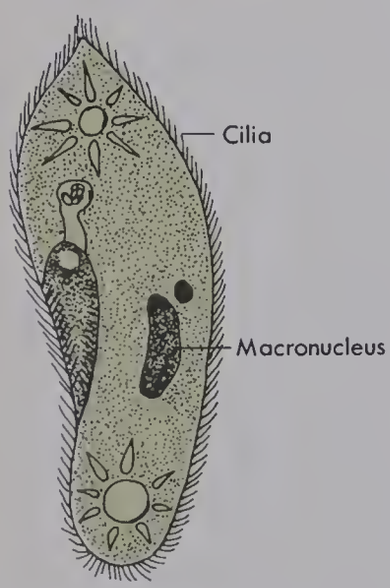


Fig. 34.16 *Paramecium*.

The Slime Molds. The members of this group are popularly known as slime molds because at one stage in their life cycle they consist of a spreading slimy mass, the **plasmodium.** The plasmodium, which contains thousands of nuclei, moves slowly over the substrate (e.g. a rotten log)

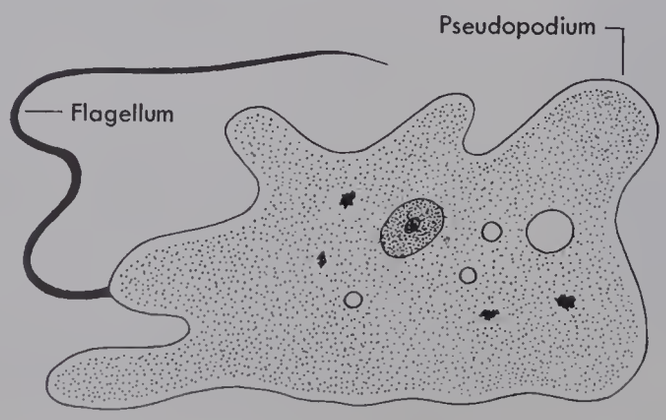


Fig. 34.17 *Mastigamoeba* moves by means of pseudopodia and a flagellum.



Fig. 34.18 *Stemonitis,* a common slime mold. Left: the plasmodium stage just prior to the formation of sporangia. (Courtesy of Prof. I. K. Ross.) Right: fully developed sporangia. (Courtesy of General Biological Supply House, Inc.)

engulfing food and growing as it does so. Eventually, the plasmodium develops elaborate stalks that produce and release spores (Fig. 34.18). If the spores land in a suitable location, they germinate to form single cells which move by both flagella and pseudopodia. These fuse in pairs and start the formation of a new plasmodium.

In other slime molds, the plasmodium is formed by the coming together of thousands of individual, amoebalike cells rather than by the growth of a single pair of cells. Experiments have shown that the individual cells are attracted to each other by the cyclic AMP (Fig. 25.12) that they release. In the course of their life cycle, these curious organisms are unicellular, multicellular, funguslike (spores), and protozoalike (amoeboid). Such a collection of traits makes them of great scientific interest. With the exception of one species that causes powdery scab in potatoes, however, these organisms are of little economic importance to man.

34.8 THE FUNGI

These eukaryotes are sufficiently different from those we have been discussing that many biologists believe that the fungi belong in a phylum of their own. Fungi consist of single cells or, more often, of tubular filaments called **hyphae.** The interwoven mass of hyphae into which most fungi develop is referred to as a **mycelium.**

Fungi do not have chlorophyll and hence are heterotrophic. Their food may be derived from such sources as rich soil, manufactured food products, and the bodies of plants and animals (both dead and living). Those that live within a living host may do so mutualistically (e.g. mycorrhizal fungi) or parasitically (e.g. corn leaf blight).

Fungi disperse themselves to new locations by releasing spores. In some aquatic species, these swim by means of flagella. However, the spores of the many terrestrial fungi are wind-blown. They are so light and are produced in such numbers that they are present almost everywhere. The spores of the wheat rust fungus have been found 14,000 feet in the air and more than 900 miles from the place where they were released.

There are some 30,000 species of fungi known. The group, which includes the molds, mushrooms, and yeasts, is divided into a number of classes of which three are of special interest.

The phycomycetes

The **phycomycetes** produce their spores in sporangia which develop at the tips of the hyphae. These spores are produced asexually; that is, two parents are not needed for the process. In the case of the water molds, the spores are flagellated and swim to new locations. Terrestrial phycomycetes produce airborne spores.

Some phycomycetes are parasitic on such organisms as fish and plants. Most are simply **saprophytes** living on dead remains of once-living organisms. They perform a valuable service in so doing because they aid the bacteria of decay in disposing of the dead and releasing their materials for reuse. Of course, the decay process can sometimes be a nuisance when valuable food products are involved. The common black bread mold (*Rhizopus nigricans*—Fig. 34.19), for example, grows upon bread or other carbohydrate-rich substrate from which it gets its nourishment.

The basidiomycetes

The fungi of the class **basidiomycetes** are dispersed by spores borne at the tips of club-shaped structures called basidia (Fig. 34.19). This class includes the mushrooms, shelf fungi, puffballs, rusts, and smuts.

The familiar mushroom or toadstool is only a portion of the fungus body. The main part of the mycelium grows beneath the surface of the ground. Only when conditions are suitable does the mycelium send mushrooms up above the surface. These are simply masses of interwoven hyphae. The basidia develop on the under sides and release their spores (four from each basidium) into the air.

The basidiomycetes are of great economic importance to man. Mushrooms are widely used for food and their cultivation is an important business. Many wild mushrooms are edible, too, but unless one takes the trouble to learn to recognize the individual species, it is best to leave them alone. A few species such as *Amanita muscaria* are extremely poisonous.

Although some basidiomycetes are good to eat, they never compensate for the food losses caused by their cousins, the smuts and rusts. The latter, especially, are responsible for serious losses to such important crops as wheat, oats, and rye.

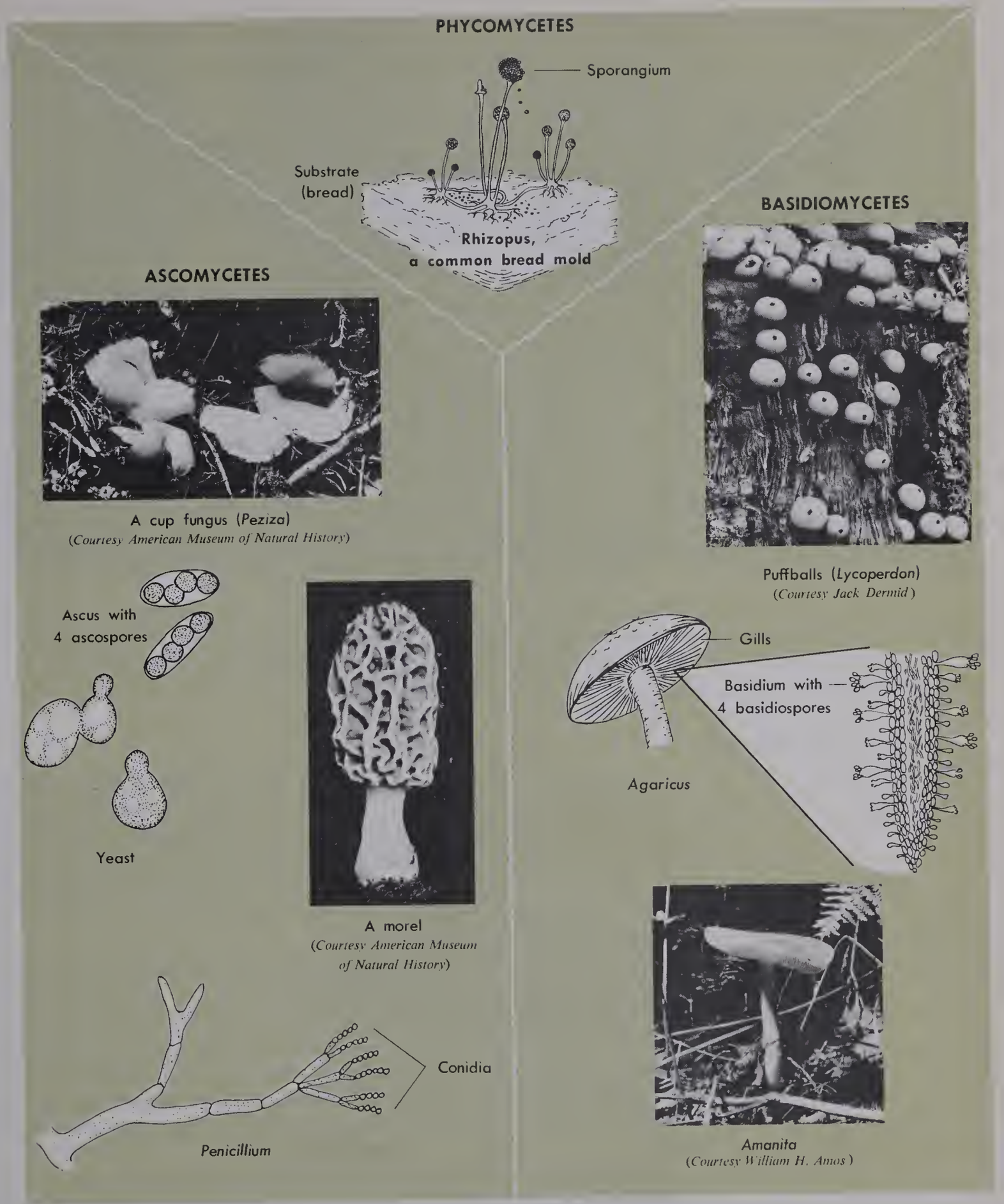


Fig. 34.19 Representative fungi.

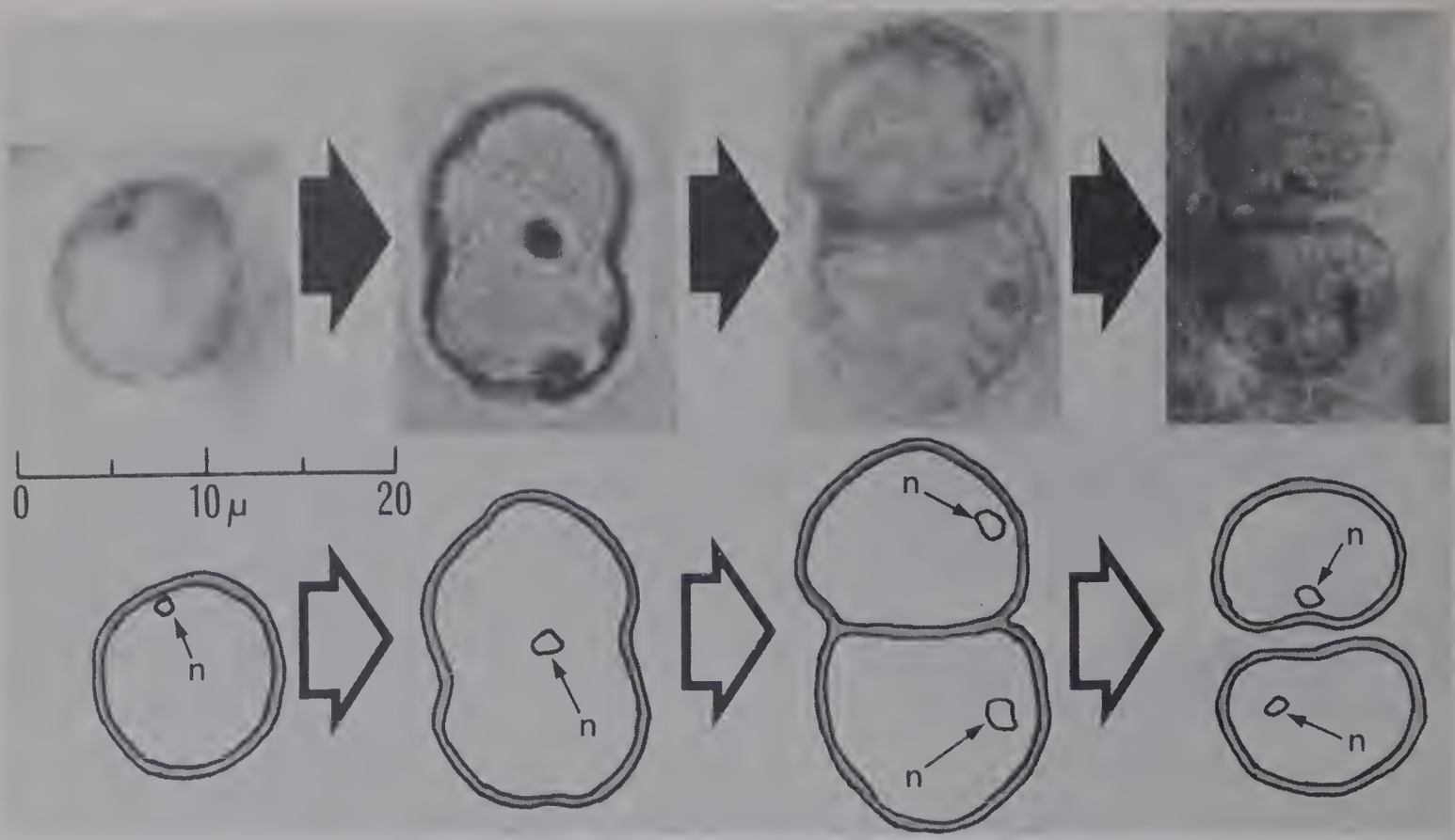


Fig. 34.20 Fossil eukaryotic algae from Australia. These fossils are one billion years old. The four photomicrographs are thought to be of the same species (named *Glenobotrydion aenigmatis*) seen at different stages of mitosis (n = nucleus). (Courtesy of J. William Schopf.)

The ascomycetes

Ascomycetes produce two kinds of spores. Those that are formed asexually, called **conidia,** develop in chains at the tips of the hyphae (Fig. 34.19). These spores are equivalent to the spores produced by the sporangium of the phycomycetes. A second kind of spore is produced as a result of sexual reproduction. Four or eight of these spores, the **ascospores,** develop inside a saclike **ascus.** We studied the life history of *Neurospora,* a typical ascomycete, in Chapter 19.

The ascomycetes play many important roles in our lives. On the deficit side of the ledger, they attack many valuable plants. The powdery mildews, which belong to this class, parasitize a variety of crop and ornamental plants. The chestnut blight is caused by an ascomycete. This organism has literally eliminated from our country what just a few decades ago was one of our major forest trees. The Dutch elm disease, also caused by an ascomycete, shows promise of doing the same to our stately American elms.

On the asset side, the ascomycete *Penicillium* produces the life-saving antibiotic penicillin. Other species in the same genus are used in the production of Camembert and Roquefort cheeses. The tasty morel, which is often thought to be a mushroom, is actually an ascomycete (Fig. 34.19).

So, too, is the truffle, considered by some to be one of the world's great delicacies.

Yeasts are unicellular members of this class. They are of enormous commercial value not only as a source of alcoholic beverages (beer, wine, etc.) but as a source of alcohol for industrial purposes. The same reaction is exploited in the baking industry, but here the desired product is the gas, not the alcohol. The carbon dioxide makes bread and cakes "rise" and gives them a spongy, palatable texture. The alcohol produced by the yeast evaporates during the baking process. Yeast is also used in the commercial production of vitamins.

The "imperfect" fungi

The ascomycetes and basidiomycetes are distinguished by the type of sexual spore they produce, ascospores in the first case, basidiospores in the second. However, many of these fungi cannot produce sexual spores unless two different strains are present to serve as the two parents. There are several thousand fungi of which only one strain is known or which for some other reason fail to reproduce sexually. In this situation, there is no way of telling whether the fungus is an ascomycete or a basidiomycete. Consequently, it is placed in a special "dustbin" category, the **fungi imperfecti.** The parasites which cause ringworm and athlete's foot in man are so classified. It is significant, however, that from time to time the sexual stage of one of these "imperfect" fungi is discovered and then it is immediately reclassified, usually to the ascomycetes.

When did the eukaryotes first appear on earth? Fossil evidence (Fig. 34.20) suggests that it was some 1 to 1.4 billion years ago, two billion years after the appearance of the prokaryotes. For another 600 million years, these organisms were undoubtedly confined to aquatic environments: the oceans and fresh water. Some 400 million years ago, however, the first eukaryotes capable of living on land made their appearance. These were the plants.

EXERCISES AND PROBLEMS

1. Of 10 gm of U-238, how much would be left after 9 billion years?
2. Some biologists have maintained that virus-like particles were the first form of life on earth. What evidence supports this hypothesis? What evidence weakens it?
3. Did Pasteur prove that the spontaneous generation of life has never occurred and does never occur? Explain.
4. How many items of evidence can you think of that suggest that all organisms on earth arose from a single first form of life?

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35 THE EVOLUTION OF PLANTS

The Geological Eras

The First Vascular Plants

The Early Pteropsids

The Mosses and Liverworts (Bryophyta)

The Angiosperms

35.1 THE GEOLOGICAL ERAS

A detailed fossil record extends back only 600 million years, a scant one-fifth of the time that life has existed on the earth

Fossils of ancient prokaryotes such as *Eobacterium* (Fig. 34.5), which is approximately three billion years old, and eukaryotes such as *Glenobotrydion* (Fig. 34.20), which is some one billion years old, are exceedingly rare. It is not until we examine sedimentary rocks formed about 600 million years ago, at the start of the Paleozoic era, that we find a wide variety of fossil organisms. This means that through more than four-fifths of the unimaginable span of time that life has existed on this earth, it left little or no record of its presence. Since that time, the record of evolving life has been quite well preserved.

The geological and biological history of the earth since the first appearance of abundant fossils is divided into three major eras (Fig. 35.1). Each of these is further subdivided into periods. At first glance it might seem surprising that marked geological changes in the earth should coincide with marked changes in the species present. Consider, though, that changes in geology (e.g. mountain formation, lowering of the sea level) bring changes in climate, and both of these alter the habitats available for living organisms. The forces of natural selection must surely have changed when the geology of the earth changed. As you can see in Fig. 35.1, the dates of the various periods are not precisely known. Only a few of the rocks (e.g. those of the early Permian) contain enough uranium to be dated accurately. The others must be dated on the basis of their relative thickness, and we have already noted the uncertainties of this method.

As we discuss the history of evolutionary change in this chapter and those that follow, you will want to refer frequently to Fig. 35.1 to see how the details fit into the whole panorama of life.

35.2 THE FIRST VASCULAR PLANTS

Although direct fossil evidence is lacking for some, we believe that almost all of the groups of organisms discussed up to this point appeared in the waters of the earth in the Pre-Cambrian period, before the start of the Paleozoic era (Fig. 35.1). During the Cambrian and Ordovician periods, the oceans and bodies of fresh water must have continued to support an elaborate array of protists and (as we shall see in the next chapter) aquatic invertebrates. But the land must have presented a desolate scene with few, if any, autotrophs adapted to life there and hence no producers to supply energy for the development of food chains. Where sea met land, however, green algae may have been evolving features that would enable them to survive intermittent periods of dryness. By the Silurian, descendants capable of life on dry land had appeared and begun to colonize this new environment. These were the plants.

The earliest plants were tracheophytes

Although the anatomy of the first plants is not entirely clear, the earliest fossils indicate that these organisms had already evolved a vascular system for the transport of water and food throughout the plant. We

Fig. 35.1 The history of life as revealed by the fossil record. ▸

ERAS	PERIODS	EPOCHS		AQUATIC LIFE		TERRESTRIAL LIFE
		with approximate starting dates in millions of years ago				
	Quaternary 0.5–3	Recent			AGE OF MAMMALS	Man in the New World
		Pleistocene		*Periodic glaciation*		First men
Cenozoic 63 ± 2	Tertiary 63 ± 2	Pliocene		*Continental drift continues*		Hominids and Pangids
		Miocene		All		Monkeys and ancestors of apes
		Oligocene		modern		Adaptive radiation of birds
		Eocene		groups		Modern mammals and herbaceous
		Paleocene		present		angiosperms
				Still attached are: N. America + N. Europe; Australia + Antarctica		
	Cretaceous 135 ± 5		AGE OF REPTILES	Modern bony fishes Extinction of ammonites, plesiosaurs, ichthyosaurs		Extinction of dinosaurs, pterosaurs Rise of woody angiosperms, snakes
				Africa + S. America begin to drift apart		
Mesozoic 230 ± 10	Jurassic 180 ± 5			Plesiosaurs, ichthyosaurs abundant Ammonites again abundant Skates, rays, and bony fishes abundant		Dinosaurs dominant First lizards: *Archeopteryx* Insects abundant First angiosperms
				Pangaea splits into Laurasia and Gondwana		
	Triassic 230 ± 10			First plesiosaurs, ichthyosaurs Ammonites abundant at first Rise of bony fishes		Adaptive radiation of reptiles (thecadants, therapsids, turtles, crocadiles, first dinasaurs) First mammals
				Appalachian Mts. formed, periodic glaciation and arid climate		
	Permian 280 ± 10			Extinction of trilobites, placoderms		Reptiles abundant (catylasaurs, pelycosaurs). Cycads and conifers; ginkgas
				Warm humid climate		
	Pennsylvanian 310 ± 10	Carboniferous		Ammonites, bony fishes	AGE OF AMPHIBIANS	First reptiles. Coal swamps
				Warm humid climate		
	Mississippian 345 ± 10			Adaptive radiation of sharks		Forests of lycapsids, sphenopsids, and seed ferns. Amphibians abundant. Land snails
				Periodic aridity		
Paleozoic 600 ± 50	Devonian 405 ± 10		AGE OF FISHES	Placoderms, cartilaginous and bony fishes. Ammonites, nautiloids		Ferns, lycapsids, and sphenopsids First gymnosperms and bryophytes First insects. First amphibians
				Extensive inland seas		
	Silurian 425 ± 10			Adaptive radiation of astracoderms; eurypterids		First land plants (psilopsids, lycapsids) Arachnids (scarpians)
				Mild climate, inland seas		
	Ordovician 500 ± 10			First vertebrates (astracaderms) Nautiloids, *Pilina*, other mallusks Trilobites abundant		none
				Mild climate, inland seas		
	Cambrian 600 ± 50			Trilobites dominant First eurypterids, crustaceans Mollusks, echinoderms Sponges, cnidarians, annelids Tunicates		none
				Periodic glaciation		
Pre-Cambrian 3000				Fossils rare but many pratistan and invertebrate phyla probably present		none

would therefore classify them today in the division Tracheophyta, the vascular plants. By the Devonian period, four distinct groups had appeared, each of which has left some descendants right up to the present time. These groups were the Psilopsida, Lycopsida, Sphenopsida, and Pteropsida (Fig. 35.2). The psilopsids, like their modern descendant *Psilotum* (Fig. 35.3), had no roots or leaves. They did, however, have both underground stems (rhizomes) and erect stems, each containing xylem and phloem. Photosynthesis was accomplished in the erect stems and these also produced the sporangia. The photosynthetic pigments were surely chlorophyll *a* and *b*, the same pigments found in their algal ancestors. Although we cannot be sure that the psilopsids were the ancestors of the other three subphyla, they certainly were the most primitive of the vascular plants. From a study of *Psilotum*, we deduce that they produced only one kind of spore, and these developed into tiny gametophytes which, in turn, produced both antheridia and archegonia. Fertilization was by swimming sperm and hence these plants must have been restricted to habitats which were quite wet, at least some of the time.

The psilopsids

The lycopsids and sphenopsids

Both the lycopsids and sphenopsids had roots and leaves, each containing xylem and phloem tissue connected with that of the stem. The leaves were simple and small, all their vascular tissue occurring in just a single, unbranched vein.

Some of the lycopsids produced not one but two kinds of spores: microspores (male) and megaspores (female). These developed into separate male and female gametophytes, respectively. In some cases, the megaspore was retained within the tissues of the parent sporophyte and, as in the gymnosperms and angiosperms of today, this provided a protected environment for fertilization to take place.

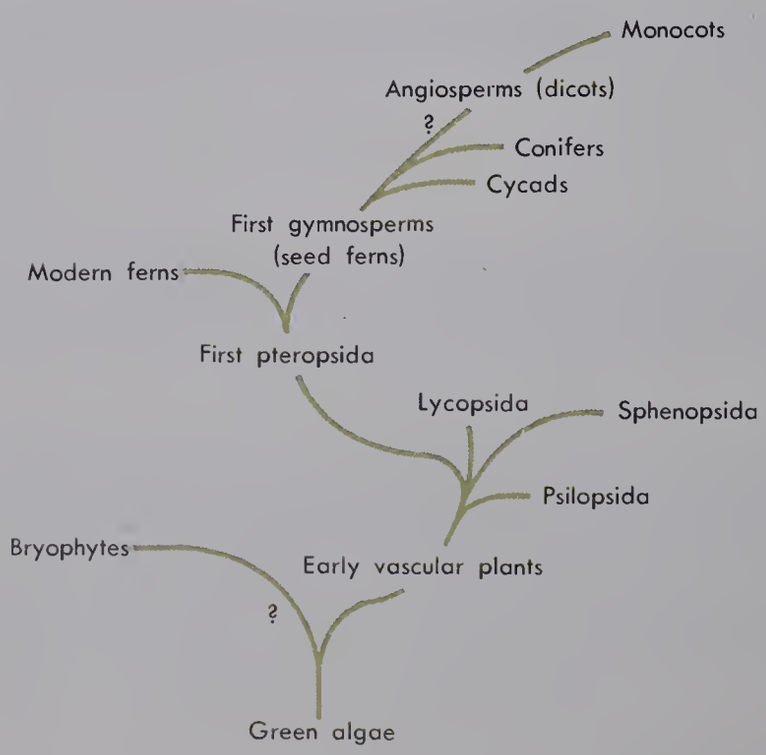


Fig. 35.2 The suspected evolutionary relationships of the plants.



Fig. 35.3 Left: a reconstruction of the Devonian landscape, showing three species of psilopsids in the foreground. (Courtesy of the Brooklyn Botanic Garden.) Right: *Psilotum nudum,* a psilopsid that still grows in Florida and elsewhere.

About 1000 species of lycopsids exist today. The genera *Selaginella* and *Lycopodium* (Fig. 35.4) are quite widely distributed. Modern sphenopsids are often called horsetails or scouring rushes. The first name is derived from their characteristic method of branching: whorls or rings of branchlets arising from the main branch (Fig. 35.5). The main branches develop



Fig. 35.4 *Lycopodium* growing close to the forest floor. Although commonly called "ground pines," they are actually members of the subphylum Lycopsida, an entirely different group. (Courtesy of William H. Amos.)



Fig. 35.5 *Equisetum palustre,* a common horsetail. It is a member of the subphylum Sphenopsida.

each season from an underground stem. The second name is derived from the fact that these plants were once used for cleaning pots and pans. Horsetails often grow in sandy locations and incorporate substantial quantities of *silica* in their stems. The silica makes the stems quite abrasive and hence an effective scouring material. The leaves are very small and are arranged in whorls or rings around the stem. Only one genus of sphenopsids, *Equisetum,* containing about 25 species, survives today.

Although the club mosses and the horsetails are practically the only modern representatives of these two subphyla, their ancestors were once many and varied and often of considerable size. Great forests of tree-sized lycopsids and sphenopsids existed during the Mississippian and Pennsylvanian periods (Fig. 35.6), and much of the world's present coal was formed by them at that time. For this reason, these two periods are sometimes referred to collectively as the Carboniferous period. When we burn this coal today, we are releasing energy stored by these plants some 300 million years ago.

Fig. 35.6 Carboniferous forest. An artist's reconstruction of what the swamp forests of the Mississippian and Pennsylvanian periods looked like. The large trunks on the left and lying flat in the foreground are of tree-sized lycopsids. The trees on the right are sphenopsids. Ferns and seed ferns are also present. (Courtesy Field Museum of Natural History.)





Fig. 35.7 Ferns. Left: tree ferns in Samoa. (Courtesy of the American Museum of Natural History.) Right: ferns of temperate climates have underground stems.

35.3 THE EARLY PTEROPSIDS

The plants in this subphylum differ from those in the other three in possessing relatively large leaves with many, often branching, veins. The subphylum is subdivided into three classes: the ferns, the gymnosperms, and the angiosperms.

The ferns

The first pteropsids, the ferns, also contributed a large array of species to the flora of the Devonian landscape. Like most of our temperate-climate ferns of today, they were **homosporous;** that is, only one kind of spore was produced. Each fern spore, you remember, develops into a prothallus that bears both male and female sex organs. Fertilization requires moisture in which the ciliated sperm can swim to the egg. For this reason, the ferns are still restricted to habitats where abundant water is present during part of the growing season. Approximately 9500 species of ferns live on the earth today. Most of these are found in the tropics, where some may grow to heights of 40 feet or more (Fig. 35.7). A smaller number are found in temperate regions. These generally grow in damp, shady locations. Their vascular roots and stem (the **rhizome**) are located beneath the ground. The leaves grow up from the rhizome each spring and manufacture food by photosynthesis. In our largest temperate species they may grow as tall as three or four feet. Although the leaves are generally killed by the first frost, the rhizome and the root system remain alive through the winter. We use



Fig. 35.8 A cycad. (Courtesy of William H. Amos.)

the term *perennial* to describe a plant which thus continues to live season after season.

Fossil remains from the Devonian indicate that some early fernlike plants were **heterosporous,** that is, they produced both microspores and megaspores. The megaspore was retained within the moist tissue of the parent sporophyte. Fertilization took place here, freed from dependence on a supply of surface water. On the other hand, the necessity for the microspores to be carried from one plant to another in order to reach the female gametophyte robbed them of their value as agents of dispersal. This function was probably taken over by seeds—dormant, protected, embryo sporophytes.

Gymnosperms were the first heterosporous plants

The seed ferns, as these plants are called, were among the earliest of the **gymnosperms.** Although seed ferns are now extinct, some of their descendants, the cycads (which resemble them closely—Fig. 35.8) and the ginkgo, survive to this day. These modern seed-bearing plants reveal their ancient lineage by the fact that after the microspore reaches the ovule, it liberates a ciliated sperm which, by swimming in moisture supplied by the parent sporophyte, reaches the egg. The ginkgo (Fig. 35.9) is the last surviving species of a once prominent group of plants. Although the ginkgo may still grow wild in the interior of China, it grows only in cultivation elsewhere. It is widely cultivated in temperate regions, however, because it grows rapidly and seems especially tolerant of smoke and other conditions of city life. The sexes are separate in the ginkgo, and male trees are generally preferred for shade-tree planting. This is because the seeds, which are produced by the female trees only, give off an unpleasant odor when crushed underfoot.

Both ferns and the early gymnosperms flourished during the Mississippian and Pennsylvanian periods. Along with the lycopsids and sphe-



Fig. 35.9 The ginkgo (courtesy of the United States Forest Service) and its leaves.

The conifers are also gymnosperms

nopsids, they contributed to the formation of coal deposits. Toward the end of these periods, the conifers arose. The microspores of these gymnosperms develop into a pollen tube, the sperm nucleus of which accomplishes fertilization. No motile sperm are produced. The conifers (e.g. pines, spruces, and firs) are the most abundant of the modern gymnosperms, but they dominate the landscape only in regions where the winters are very cold. This group includes the largest and oldest of all living organisms. One redwood (genus *Sequoia*) growing in California is 367 feet high. Bristlecone pines growing high in the mountains of eastern California have been shown to be over 4000 years old.

35.4 THE MOSSES AND LIVERWORTS (BRYOPHYTA)

Some 23,000 species of living mosses and liverworts have been identified. These are small, fairly simple plants that are usually found growing in moist places. The liverworts have a thin, leathery body that grows flat upon the supporting medium—still water or moist soil. Figure 35.10 shows a common liverwort growing on the surface of pond water.

Bryophytes have no xylem, phloem, or cambium

The plant body of mosses is a little more elaborate than that of liverworts. It consists of an erect shoot bearing tiny leaflets arranged in spirals. Neither mosses nor liverworts have any woody tissues for support and thus they never grow very large. They have no specialized vascular system for the transport of water and food throughout the plant.



Fig. 35.10 Representative bryophytes. Left: the common haircap moss *Polytrichum commune.* (Courtesy of the American Museum of Natural History.) Right: a common liverwort *Ricciocarpus natans.* (Courtesy of William C. Steere and *AIBS Bulletin.*)

In Chapter 20 we learned that sexual reproduction in mosses can occur only if the sperm cells are able to swim or be splashed from the plant that produces them to the plant where the egg is. The lack of a special water transport system and the need for free water for sexual reproduction are two reasons why the bryophytes are restricted to habitats that, periodically at least, have abundant moisture.

Bryophytes are probably not "primitive"

The bryophytes have sometimes been considered the ancestors of the vascular plants. Their simplicity of structure, lack of vascular tissue, and restriction to damp locations do suggest that they are intermediate forms between the algae and the vascular plants. The fossil record indicates, however, that this interpretation is false. No bryophyte fossils are found in rocks formed before the Devonian period, but as we have seen, vascular plants were already present in the Silurian. We may conclude, then, that the mosses and liverworts either represent a second, rather unsuccessful, colonization of the land by aquatic ancestors or possibly (as some have suggested) are descendants of land plants that have lost many of the adaptations of their ancestors. In either case, the lack of a vascular system and woody tissue and the necessity for surface water in which the sperm can move from antheridia to archegonia have limited the evolutionary potentialities of these organisms.

35.5 THE ANGIOSPERMS

Although the first angiosperms appear in the fossil record in Jurassic deposits, it was not until the end of the Mesozoic era that they became the

dominant plants of the landscape. That they are the dominant plants on earth today is unquestionable. There are some 200,000 species of living angiosperms. The rest of the plant kingdom includes only about 34,000 living species. The angiosperms are found in practically every habitat. Although they incorporate a variety of features that enable them to live even in arid locations, some have returned to an aquatic existence (Fig. 35.11).

Dicots vs. monocots

The angiosperms are divided into two subclasses, the dicots and the monocots. These names are derived from the number of cotyledons present in the seeds: two in those of the first group, one in those of the second. Dicots and monocots also differ in several other respects (Fig. 35.12).

The dicots are the larger (and older) of the two groups, with approximately 150,000 species known. The buttercup, snapdragon, carnation, magnolia, poppy, cabbage, rose, pea, poinsettia, cotton plant, cactus, carrot, blueberry, mint, tomato, sunflower, elm, oak, and maple represent 19 of the 250 families of dicots.

About 50,000 species of monocots are known. These include the lilies, palms, orchids, irises, tulips, sedges (a grasslike plant of marshy locations), onions, asparagus, and the grasses. The grasses include corn, wheat, rice, and all the other cereal grains upon which we depend so heavily for food.

To what can we attribute the great success of the angiosperms? Probably the answer is that they incorporate the most efficient adaptations to dry-land living with the most varied and efficient methods of sexual reproduction and dispersal. Let us review the features which have permitted angiosperms (and, in varying degrees, other plants) to invade the dry land.



Fig. 35.11 A common "duckweed," one of the smallest angiosperms.

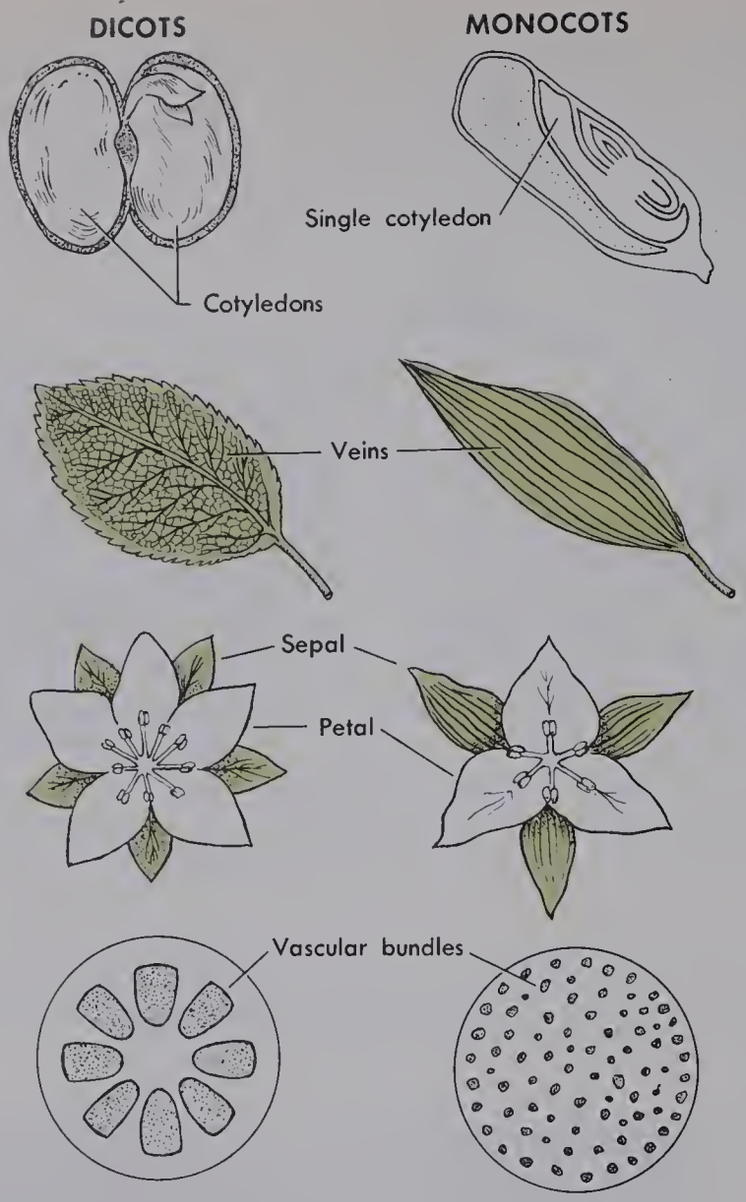


Fig. 35.12 A comparison of the structural patterns found in dicots and monocots.

Adaptations to land living found in the angiosperms

The possession of roots permits the extraction of moisture and minerals from beneath the surface of the land. Roots also serve to anchor the plant against the wind. The presence of a cambium capable of producing woody tissue provides for the support, high in the air if need be, of leaves and flowers. Xylem and phloem enable water, foods, hormones, etc., to be translocated long distances in the plant quickly and efficiently. A waxy cuticle on leaves and herbaceous stems and the cork on woody stems prevent rapid loss of water from the plant by evaporation. Such a waterproof covering is also gasproof, but the needs of gas exchange are taken care of by stomata and lenticels. The shedding of leaves in most temperate-climate angiosperms further reduces loss of water during the winter (when soil water may be frozen) and also helps reduce the likelihood of mechanical damage to the plant from the accumulation of ice and snow.

The angiosperms share with the gymnosperms the characteristic of retaining the female gametophyte within the megasporangium. As we have seen, this device puts an end to the need for surface water to effect

the transfer of sperm from one plant to another. Instead, pollen grains can be transported by air to the megasporangium, thus bringing the gametes together. In the gymnosperms and some angiosperms, the pollen grains are simply blown from plant to plant by the wind. Many other angiosperms attract insects or other animals by their flowers and thus exploit the animal's power of locomotion to aid cross-pollination. The quantity of pollen produced by animal-pollinated angiosperms is usually far less than that produced by wind-pollinated species.

Functions of the seed

The embryo sporophyte plant, the seed, is also an effective adaptation to dry-land living. Protected by seed coats and supplied with food reserves, the seed can withstand harsh, dry conditions for long periods while still remaining ready to germinate when conditions finally become suitable. Seed production in the angiosperms is more efficient than in the gymnosperms in that food is translocated into the seed only if fertilization has taken place. In the gymnosperms, the food reserves of all the potential seeds are deposited before fertilization and thus wasted if pollination should fail to occur.

The significance of flowers and fruits

Most of the adaptations mentioned so far are not unique to the angiosperms. They have played a role in allowing the other plants to colonize the land, too. It is only in the production of **flowers** and **fruits** that the angiosperms stand alone. The efficient methods of pollination and seed dispersal which flowers and fruits make possible, added to all the other features which reach their fullest development in the angiosperms, have enabled these plants to invade nearly every conceivable habitat on this earth. In the angiosperms, we see the most adaptable group of plants yet produced by evolutionary change in organisms that were once restricted to an aquatic world.

The colonization of the land by vascular plants in the Silurian had far-reaching consequences. Plants are autotrophic and their presence on land provided there for the first time an abundance of food for heterotrophic organisms. The fungi and many animals were quick to exploit this and soon followed the plants in adapting to the special conditions of dry-land living. In the next chapters we shall examine how animals accomplished this transition. It will be an important part of our larger story of the evolution of the animals as a whole.

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36 THE EVOLUTION OF THE INVERTEBRATES

Introduction

The Sponges (Phylum Porifera)

The Cnidarians (Phylum Cnidaria)

The Flatworms (Phylum Platyhelminthes)

The Origin of Animals

The Roundworms (Phylum Aschelminthes)

The Annelid Worms (Phylum Annelida)

The Mollusks (Phylum Mollusca)

The Arthropods (Phylum Arthropoda)

The Echinoderms and Chordates

36.1 INTRODUCTION

Animals are organisms that (1) do not have chlorophyll, (2) are capable of locomotion or, at least, body movement by means of contracting fibers, and (3) are multicellular. A few organisms fail to meet the second criterion but show such close similarities to creatures that do, that we cannot fail to recognize them as animals.

Invertebrates vs. vertebrates

The animal kingdom is generally divided into some 20 to 24 different phyla. The animals that make up all but one of these have no backbone and hence are commonly described as invertebrates. One phylum (our own) contains a few invertebrates, and these will be discussed in this chapter. The remaining members of the phylum are the extraordinarily successful vertebrates. Their evolution will be studied in the following chapter.

Three of the animal phyla include creatures built on rather simple patterns of organization. For this reason, we suspect that these animals are primitive, or to put it another way, are little-changed descendants of the earliest forms of animal life. These animals are the sponges, the cnidarians, and the flatworms.

36.2 THE SPONGES (PHYLUM PORIFERA)

These are simple animals that spend their lives anchored to a rock or other solid surface underneath the water. About 4000 species are known, a few of which live in fresh water, but most live in the ocean. The phylum gets its name from the many small openings or pores that perforate the sponge body. The animals feed by drawing water in through these pores and filtering out tiny food particles that may be present (Fig. 36.1).

The sponge body consists of two layers of cells with a layer of jellylike material, the **mesoglea,** between them. The cells of the inner layer have flagella which set up the water currents. These cells also consume the filtered food particles.

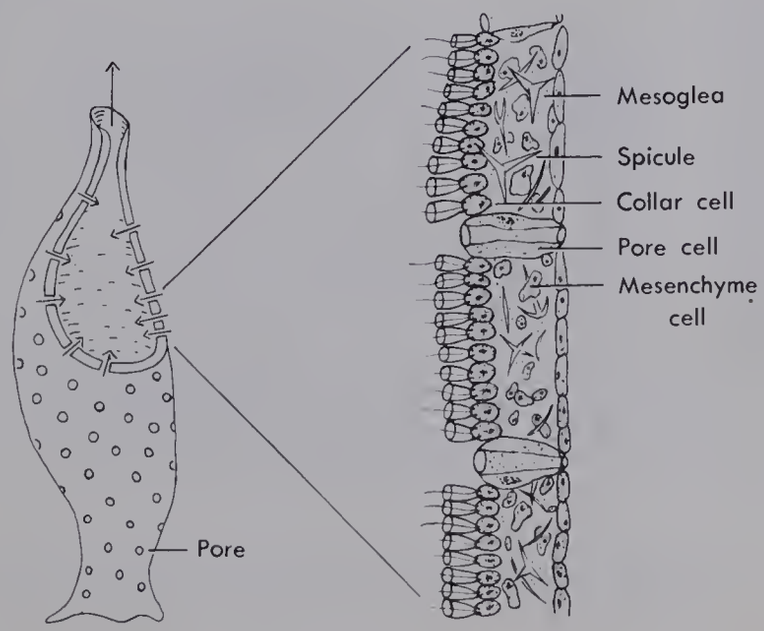


Fig. 36.1 Structure of a simple sponge. Sponges secure food and oxygen from the water which is continuously drawn in through their pores.

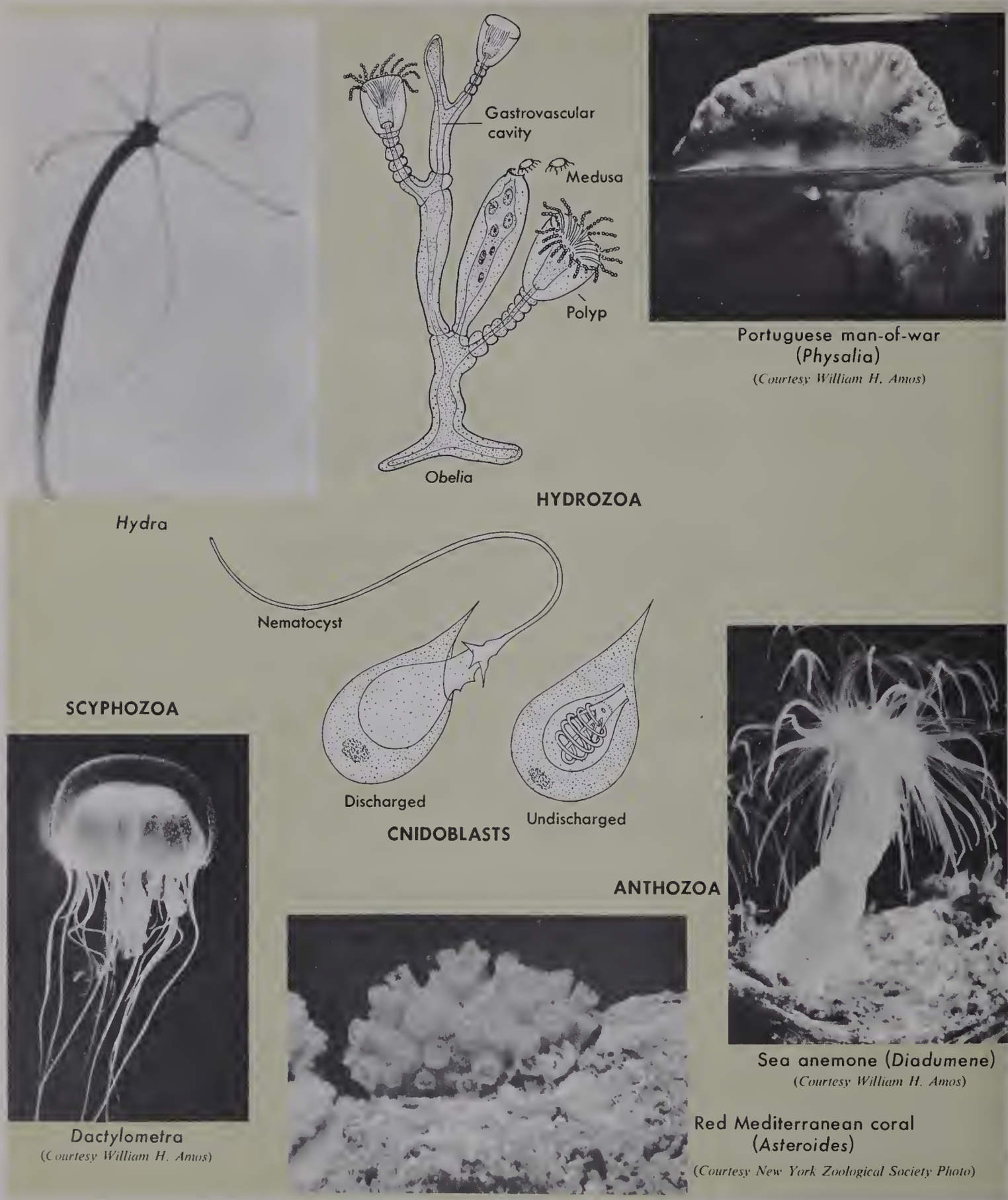
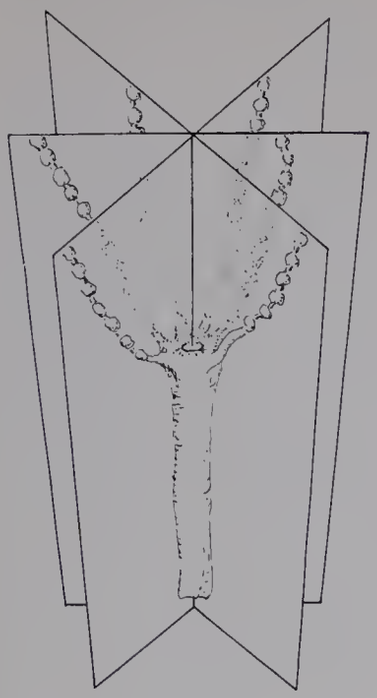


Fig. 36.2 Representative cnidarians. Most of the members of this phylum live in salt water.



The shape of the sponge is maintained by a skeleton of spicules formed by scattered cells in the mesoglea. The spicules are quite hard, being composed of either silica or limestone (calcium carbonate). Some sponges have no spicules but instead are supported by a network of flexible, tough fibers. These sponges, which occur in shallow tropical waters, are harvested by divers and after processing are sold for cleaning purposes.

Organisms that are anchored to one spot must have some means of dispersing their offspring to new locations. The sponges accomplish this by producing small, free-swimming **larvae.** These swim away from the parent sponge and after finding a suitable new surface, attach themselves to it and develop into adults.

Fossil remains indicate that sponges were one of the earliest forms of animal life to appear on earth. There is no evidence, however, that any other animal forms have evolved from sponges since then. The sponges seem to occupy a rather unique place in the animal kingdom and have, in fact, been placed in their own subkingdom, Parazoa, by some taxonomists.

36.3 THE CNIDARIANS (PHYLUM CNIDARIA)

All the members of this phylum possess specialized stinging cells called **cnidoblasts** that have given rise to the phylum name. Each cnidoblast contains a poison-filled, barbed thread, the nematocyst (Fig. 36.2). When the trigger of the cnidoblast is touched, the nematocyst is discharged. It is used for trapping and paralyzing prey as well as for defense against enemies.

The body of all members of this phylum consists of two layers of cells with a jellylike mesoglea between them. The mesoglea has cells scattered through it, however, and is considered by some biologists to be a third cell layer. The body is organized as a hollow cylinder with a single opening at one end. Food is introduced through this opening, the mouth, and into the inner cavity, called the **gastrovascular cavity.** This cavity is also called a coelenteron and for many years the name of this phylum was Coelenterata. Another group of animals (called comb jellies) were included then as they also have a coelenteron. They have no cnidoblasts, however, and are no longer thought to be closely related to the forms we are discussing.

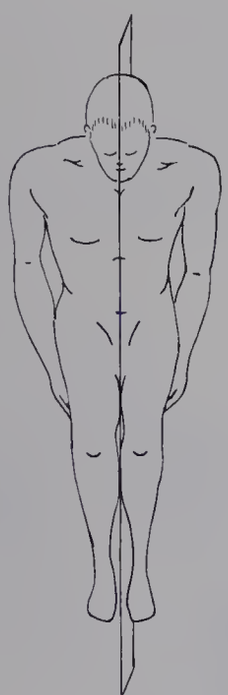


Fig. 36.3 Bilateral symmetry is characteristic of animals that move about actively. They have right and left sides, a front and a back. Animals with radial symmetry spend their lives anchored to one spot or move about quite sluggishly.

All the parts, such as tentacles, in the Cnidaria are arranged in a circle around the cylindrical body. This pattern of organization is known as radial symmetry. If we should cut a hydra from the head (anterior) to the base (posterior) in any plane passing through the midline, the organism would be divided into two equal halves. Contrast this with the bilateral symmetry of man. There is only one plane passing through the midline that will divide the human body into two equal halves. This is the plane running from the back (dorsal) surface to the front (ventral) surface (Fig. 36.3). It divides the body into right and left halves. Radially symmetrical animals like the cnidarians have neither dorsal and ventral surfaces nor right and left sides.

About 9600 species of Cnidaria are known. Most of these are found in the oceans, although a few species, such as *Hydra* (Fig. 36.2), occur in fresh water. The phylum is divided into three classes.

Hydrozoa

The ready availability of the common fresh-water hydra makes it the most common cnidarian studied by biology students. Although it exhibits the major structural features of the phylum, it is really not typical of either its phylum or its class (Hydrozoa). First of all, unlike most of the other members of the phylum it is found in fresh water. Second, it exists as a single individual. Most members of its class are colonial, that is, many individuals live attached to one another. Third, *Hydra* exhibits only one body form, the **polyp** (Fig. 36.4). Most members of the class produce a second body type, the **medusa,** that floats or swims freely in the water and aids in the dispersal of the species. Despite its superficial difference in appearance, the medusa is basically an upside-down polyp. Figure 36.2 shows the two body forms as they occur in *Obelia,* a more nearly typical member of the class. The Portuguese man-of-war *(Physalia),* whose nematocysts may cause serious, even fatal, poisoning in man, is also a member of this class. It consists of a floating, gas-filled bag from which dangle long chains of polyps.

Polyp vs. medusa

Scyphozoa

The main body form of jellyfishes (cnidarians of the class Scyphozoa) is the medusa. Their jelly is simply a greatly enlarged mesoglea. The tentacles of the medusa bear the cnidoblasts, which in some species can cause considerable pain to unwary swimmers. Sea anemones and corals (which belong to the class Anthozoa) consist of a polyp stage only. The corals secrete limestone shelters that are responsible for the development of great reefs and atolls in tropical waters.

Anthozoa

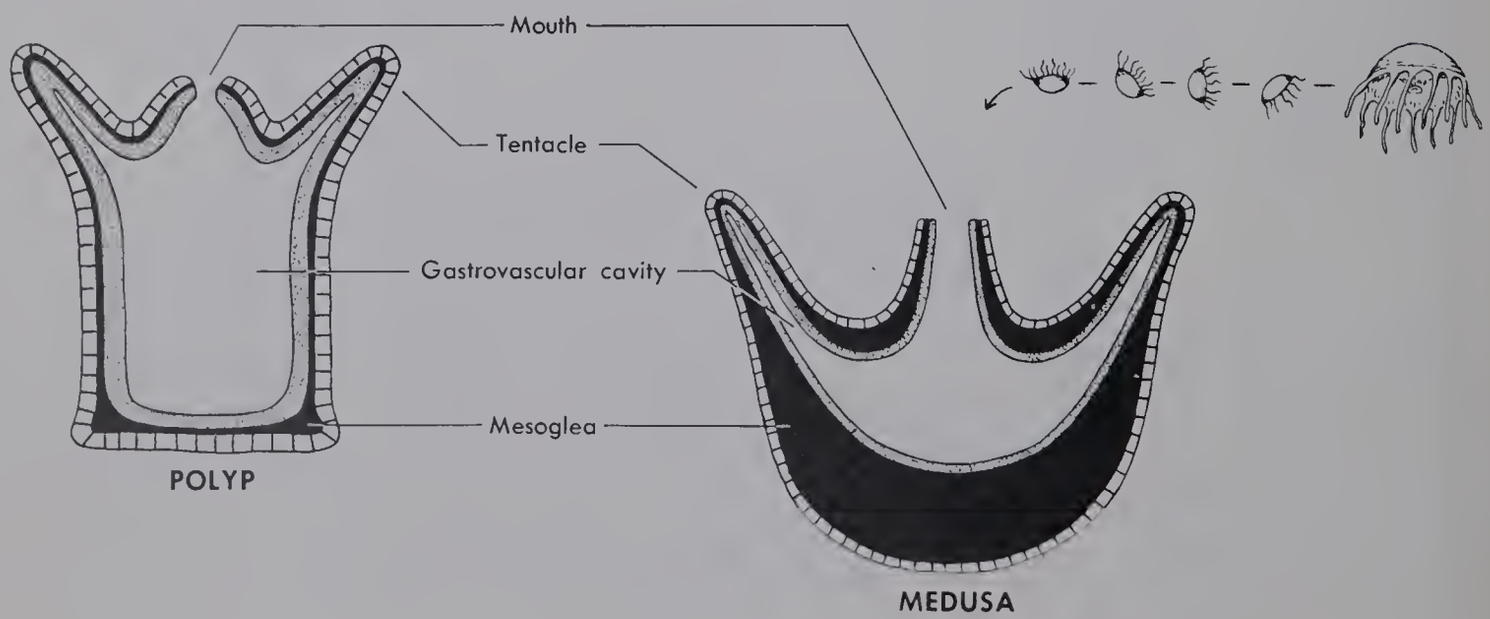


Fig. 36.4 The two body forms found in cnidarians. The medusa has been turned upside down to show its basic similarity to the polyp. The jelly of a jellyfish (medusa) is a greatly enlarged mesoglea.

36.4 THE FLATWORMS (PHYLUM PLATYHELMINTHES)

This phylum contains some 7000 species. These organisms are aptly named: many are almost ribbonlike in shape. They are bilaterally symmetrical with right and left sides and a dorsal and ventral surface as well as an anterior and posterior. This type of symmetry seems to be associated with active locomotion. Many of the common freshwater flatworms, called **planarians,** can move quite rapidly. When attached to an underwater surface, they secrete a slimy mucus layer underneath them and then propel themselves forward on the mucus by the beating of myriads of cilia on their ventral surface. When suspended freely in the water, planarians swim by undulating body movements. The efficient locomotion of the planarians enables them to seek their food actively, in contrast to the radially symmetrical cnidarians.

Bilateral symmetry is also associated with a concentration of sense organs at the anterior end of the animal. Planarians have light, touch, and vibration receptors at their anterior end, the end that meets changes in the environment first. Such a concentration of sensory equipment in the head is called **cephalization.**

The food of the planarian is taken in through a mouth on the ventral surface and into a gastrovascular cavity. Although this cavity is much more elaborate in shape than that of the hydra, it is built upon the same saclike plan. Undigested material must still leave by the mouth as it does in the hydra.

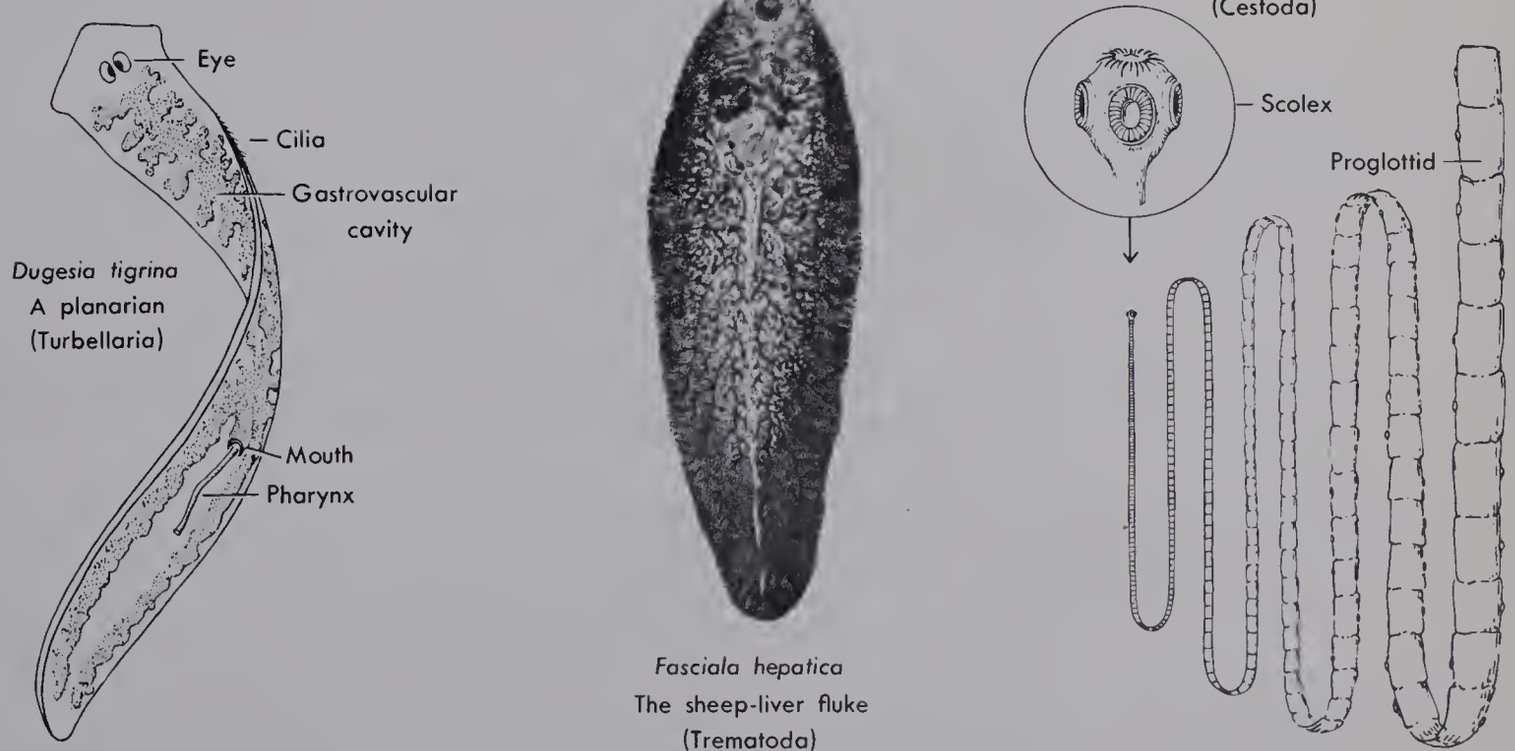


Fig. 36.5 Representative flatworms (Platyhelminthes). The tapeworm has no digestive organs. (Photo courtesy of the General Biological Supply House, Inc.)

Turbellaria

Many of the free-living flatworms (Class Turbellaria) are not nearly as complex as the common freshwater planarians that we have been discussing. In fact, some of them are constructed so simply that biologists feel they are the most primitive bilaterally symmetrical animals and perhaps all the other phyla we shall discuss evolved from early members of this group. Today these worms can be found in moist soil and salt water as well as in fresh water.

Flukes are assigned to the class Trematoda

Two classes of flatworms are composed exclusively of parasitic forms. Adult **flukes** (Fig. 36.5) remain attached to their host by means of suckers on the ventral surface. Many produce larvae that are also parasitic but in a different host, usually some species of snail. Blood flukes and lung flukes, as well as liver flukes, are serious parasites of man, particularly in some of the warmer regions of the earth.

The tapeworm class is Cestoda

Tapeworms, like flukes, are parasitic. The adults live in the intestine of their hosts and absorb nourishment from their surroundings. They form ribbonlike colonies of relatively independent proglottids (Fig. 16.2). These may grow to lengths of 30 feet or more.

Most tapeworms need two or more different hosts in order to complete their life cycle. Common tapeworms of man are acquired by eating infected, undercooked fish, beef, and pork.

36.5 THE ORIGIN OF ANIMALS

From what did the animals evolve? We simply do not know. The event occurred in pre-Cambrian times and no fossils that might enlighten us have been found. Thus we are forced to examine evidence such as homologous organs, homologies in the pattern of embryonic development, even biochemical homologies—all in living forms—in order to provide a basis for our speculations.

Animals may have evolved from colonial protozoans

While a number of theories of animal origins have been proposed, they fall into two rather distinct categories. On the one hand, there are those who believe that the multicellularity characteristic of animals arose as a consequence of the formation of colonies of cells. With the development of specialized cells within the colony, interdependence of parts follows, and the boundary between a colony and a multicellular individual has been crossed. In the sponges the presence of collar cells, which closely resemble certain unicellular flagellates (Fig. 36.6), suggests that sponges did evolve this way.

What about the other animals? Perhaps they, too, arose this way, but independently of the sponges. Some biologists think that colonial green algae were not only the ancestors of the plants but also the ancestors of the animals. This theory is supported by the existence of larval cnidarians whose structure—a ciliated, hollow ball of cells—is suggestive of the structure of *Volvox*. Inward migration of some of the surface cells of this larva produces two tissue layers, and later the gastrovascular cavity of the mature cnidarian develops within. The flatworms, which also have a gastrovascular cavity with a single opening, are considered descendants of the

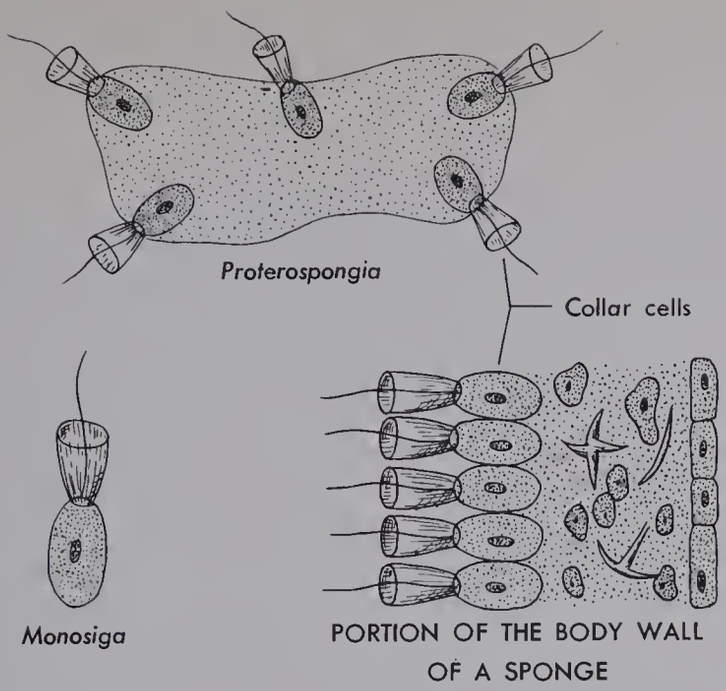


Fig. 36.6 The presence of collar cells in sponges suggests that these multicellular animals have evolved from colonial forms like *Proterospongia*. They, in turn, probably evolved from unicellular forms.

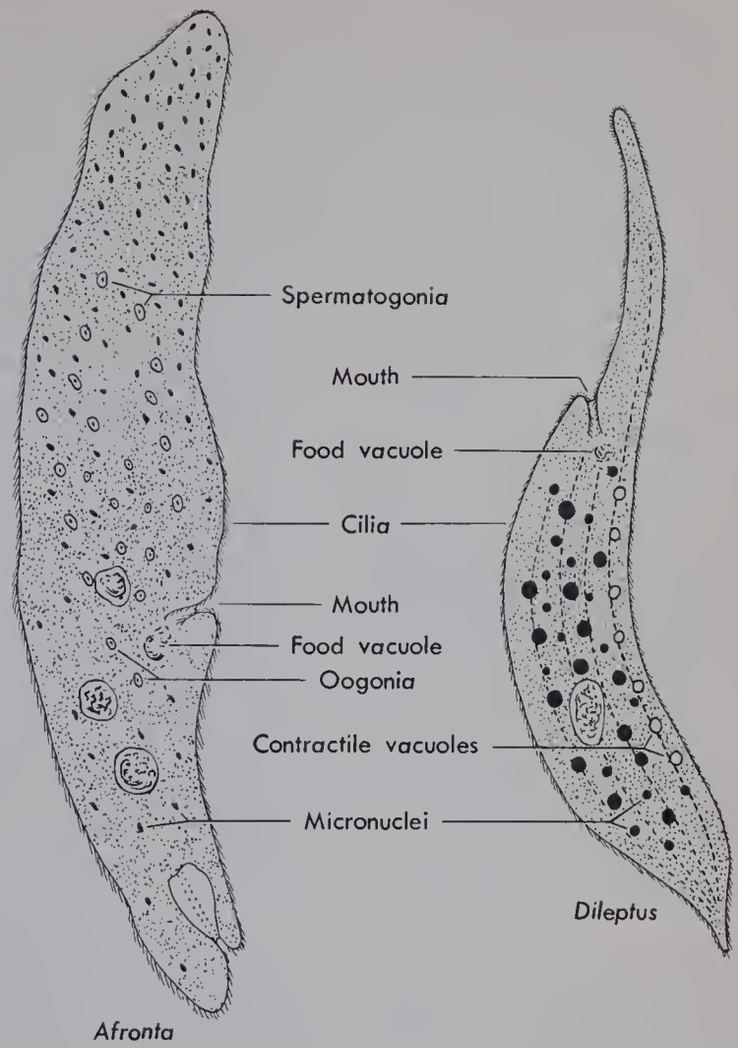


Fig. 36.7 A syncytial flatworm (left) compared with a ciliated protozoan (right). The only true cells in *Afronta* are the oogonia and spermatogonia.

cnidarians. (One wonders, though, what happened to that most distinctive feature of the cnidarians, the cnidoblast. It is true that some flatworms have cnidoblasts, which they use as weapons, but they do not grow them. They simply incorporate them into their own epidermis after first securing them by eating hydras!) With the penetration of the gastrovascular cavity through the other end of the organism, the tube-within-a-tube body plan of the other animals could have been formed.

Animals might have evolved from multinucleated ciliates

An alternative explanation for the orgin of animals is that they arose by the cellularization of ciliated protozoans. As we noted in Chapter 34, many ciliates are quite large (for single cells) and exceedingly complex in their anatomy. Often these forms have a large number of nuclei scattered through the cytoplasm. Developing an internal system of membranes to partition these nuclei into separate cells would transform these creatures into *bona fide* animals.

In fact, some tiny flatworms (relatives of the planarians) resemble ciliated protozoans in a number of ways, including size (Fig. 36.7). These worms are syncytial; that is, most of the animal body is a continuous mass of cytoplasm in which are suspended many nuclei. In this feature, and in their methods of locomotion and nutrition, these animals are very like the

ciliates and provide a plausible link with the protists. Then, from a syncytial flatworm, it is not much of a jump to more specialized forms such as the planarians.

36.6 THE ROUNDWORMS (PHYLUM ASCHELMINTHES)

Roundworms, or nematodes, are elongated, cylindrical animals. They show two evolutionary advances over the flatworms (from which they may have evolved). They have a one-way digestive tract running from a mouth at the front to an anus at the rear. This one-way digestive system is advantageous as it eliminates the mixing of incoming food with outgoing wastes. After food enters the mouth, it can be processed step-by-step as it passes from one section of the digestive tract to another. Finally, undigested remains are eliminated at the anus. Nematodes also have a cavity between the digestive tract and the body wall. It develops from the blastocoel cavity during embryonic development and is not therefore related to the coelom which, as you remember, develops totally surrounded by mesoderm. For this reason it is often called a *pseudocoel.* In it are found various internal organs including those of reproduction. The presence of a definite body cavity rather than a solid mass of mesoderm allows for greater freedom of movement although the whiplike motion so characteristic of nematodes does not seem to exploit this.

The pseudocoel develops from the embryonic blastocoel

The majority of nematodes are quite small. A few, such as *Ascaris* (Fig. 36.8), may be almost a foot in length and one, a veritable giant that parasitizes whales, reaches a length of 30 feet! Most nematodes, however, are not much bigger than small bits of thread. They can nevertheless be easily identified by the thrashing, whiplike movements they make. Rich soil literally teems with these little organisms and examination with a lens of a little soil and water is sure to reveal their presence.

About 10,000 species of nematodes have been identified so far, but the list is surely far from complete. Nematodes live practically everywhere. They are found in fresh water and salt water, as well as soil. As parasites, they live in the bodies of plants and other animals. It has been said that if all the matter of our earth disappeared except for roundworms, we would still be able to recognize all the formerly existing features—living as well as nonliving—by the nematodes they had contained.



Fig. 36.8 *Ascaris,* a parasitic nematode, in the human intestine.

Hookworms

Most nematodes are free-living. It is the parasitic forms, however, that are of greatest interest to man. One of the most serious of these in this country is the hookworm. This organism lives attached to the wall of man's intestine and sucks blood and tissue fluids from it. A heavy infestation causes great weakness and lethargy. The disease is usually contracted by walking barefoot on soil contaminated by human excrement. At one time the American hookworm infected over two million inhabitants of the southeastern part of our country. Now proper sanitation and the wearing of shoes have done much to reduce the incidence of infection.

Trichinosis

The Southeast has no monopoly on nematode infections. According to recent estimates, over 4 million people throughout the United States have been more or less heavily infected with the nematode *Trichinella spiralis.* Infection comes from eating raw or undercooked pork that contains the organisms (Fig. 33.7). One is wise to assume that all pork contains them, because the federal government neither inspects for the presence of this parasite nor forces pig growers to take the necessary precautions to avoid contamination of their animals. Infection of man by *Trichinella spiralis* is something of a biological mistake. Inasmuch as we do not practice cannibalism or leave the bodies of our dead around carelessly, there is no way for the worms to escape to another host animal. They simply form a resting stage in our muscles and ultimately die. In a heavy infection, however, the worms may cause such serious body disturbances that death of the host from **trichinosis** results.

Ascaris

Humans, especially children, are also frequently infected with other kinds of nematodes, such as *Ascaris,* whipworms, and pinworms. These all live in the intestine and are contracted by careless sanitary habits. Infestation is usually not serious, however, and can be easily cured. The "worms" for which we have the family dog treated periodically are usually also nematodes of the genus *Ascaris.* The filarial worm, which causes the gross deformity of elephantiasis, is one of several parasitic nematodes that afflict humans living in tropical regions (Fig. 13.15).

Our survey of the nematodes would not be complete without mentioning the great damage that they do to crop plants such as oranges, tobacco, and strawberries. These parasites seldom kill their host outright, but they may weaken it so that it succumbs to some other invader. Only in recent years have agricultural scientists come to realize that nematode attack may be the basic cause of a large percentage of our annual crop losses.

36.7 THE ANNELID WORMS (PHYLUM ANNELIDA)

The worms in this phylum are segmented; that is, their bodies are made up of repeating units. Although some structures, such as the digestive tract, extend the entire length of the worm, others such as the excretory organs are repeated in segment after segment. The segmentation is revealed externally as a series of rings (Fig. 36.9).



Fig. 36.9 Representative annelids. Left: an earthworm. (Courtesy of the American Museum of Natural History.) Center: a clam worm. (Courtesy of the New York Zoological Society.) Right: a leech. (Courtesy of William H. Amos.) The leech is carrying its young who are themselves being preyed upon by three small leeches of another species.

The organization of the body into segments appears to have been a significant evolutionary advance. Segmentation is a way of achieving additional size without having to increase the size or efficiency of individual organs. It is necessary only to repeat them in segment after segment. Size, in turn, has advantages. Larger organisms can cope successfully with food too large to be eaten by their smaller competitors.

Other characteristics of the annelids are bilateral symmetry, an efficient circulatory system with blood pumped through a closed system of blood vessels, and a fairly elaborate nervous system. The major nerve trunk runs along the ventral side.

Embryonic origin of the coelom

Another feature of the annelid worms not found in more primitive animals is the presence of a large fluid-containing body cavity. This permits the internal organs to slide easily against one another and this, in turn, makes extensive movements of the body easy to accomplish. The cavity, called a **coelom,** is lined throughout with mesoderm. However, its embryonic development is quite different from the way in which the coelom develops in vertebrates (see Section 22.3). Early in cleavage, special mesodermal cells are formed within the embryo. Mitotic division of these cells

produces a mass of mesoderm tissue. Eventually a space develops within this mass, and this gradually enlarges to form the coelom.

About 7000 species of annelids have been identified. The majority of these are marine, although the earthworm is a more familiar example for those of us who do not live at the seashore. Leeches are also members of the group (Fig. 36.9). The marine annelids produce free-swimming larvae called **trochophores** (Fig. 36.10).

Trochophore larvae

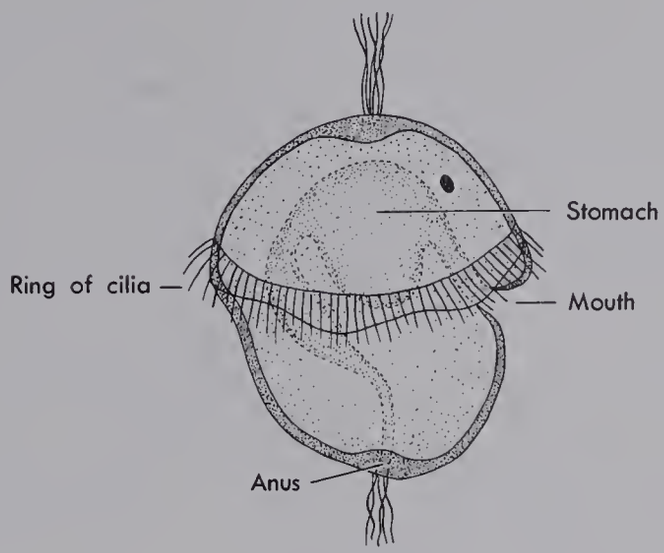


Fig. 36.10 A trochophore larva. These are produced by members of several animal phyla, including annelids and mollusks.

36.8 THE MOLLUSKS (PHYLUM MOLLUSCA)

With about 100,000 living species, this phylum must be included among the most important animal phyla on earth today. It consists of soft-bodied, unsegmented (with one exception) animals many of whom are protected by one or more shells made of limestone (calcium carbonate). These shells (or valves) are formed by a special fold of the body wall called the **mantle.** The great majority of the mollusks live in salt water, but many are found in fresh water and some occur on land. The phylum is divided into three major and several minor classes.

Bivalvia

The clams, oysters, mussels, scallops, etc., are commonly known as **bivalves** because two shells encase the body. The bivalves have bilateral symmetry, but in their case it is not associated with rapid locomotion. Those that move at all do so by extending a thick, muscular foot from between the valves. All members of the class feed by filtering food particles from water which they draw in under the mantle.

Gastropoda

A second large class of mollusks includes all the **snails** and their shell-less relatives, the **slugs.** Snails are often referred to as **univalves** because of their single shell. This shell is coiled, as are the internal organs of the animal. There is no plane of symmetry in adult snails although they develop from bilaterally symmetrical larvae. Snails feed by scraping food with a rasping, tonguelike *radula.* They have a distinct head with two eyes which are often located on stalks. The great majority of snail species live

in salt water but some are also found in fresh water and even on land. The latter breathe air by means of a lunglike adaptation of the mantle. Slugs are found in salt water and on land. Terrestrial ("garden") slugs are rather dark in appearance but marine slugs are often brilliantly colored and decorated.

Although some species are prized as food, snails cause far more food loss for man than their consumption compensates for. Many marine snails, such as the oyster drill, feed upon commercially valuable bivalves. But far more serious is the destruction brought to crops in some parts of the world by the terrestrial slugs and snails. Some snails also serve as an intermediate host for flukes. Despite the damage caused by some species, the group as a whole should be prized if only for the extraordinary array of beautiful snail shells that are cast up on our beaches, bringing delight to casual and serious shell collectors alike.

Cephalopoda

The various species of octopus and squid (Fig. 36.11) as well as the chambered nautilus belong to the class Cephalopoda. All of these organisms have a large, well-developed head bearing prominent eyes and surrounded by a ring of arms (eight in the octopus, ten in the squid) which aid in locomotion and the grasping of prey. They are found exclusively in salt water. Although the cephalopod eye looks and functions much like the vertebrate eye (such as ours), these are analogous, not homologous, organs and have arisen as a result of convergent evolution. Only the chambered nautilus has a large shell. The shell of squids is reduced to a thin plate imbedded in the mantle while octopuses have no shell at all.

The cephalopod mollusks are the most complex members of the phylum. They also include the largest of all the invertebrates. Records exist of a 28-foot octopus and a 50-foot squid. Squids have curious methods of locomotion and defense. They can move very rapidly by forcibly ejecting a stream of water from under the mantle. When in danger, the squid supplements its jet-propelled escape by squirting a black, inky fluid into the water and thus distracting its enemy. Both octopuses and squid form an important item of man's diet in some parts of the world.

Scaphopoda

Other interesting mollusks are the "tooth shells," a small class of marine mollusks that spend their adult lives almost buried in the sand. They feed by filtering tiny organisms out of the water that is drawn in through the opening in the protruding end of their shell (Fig. 36.11).

Polyplacophora

Chitons are sluggish organisms that live inconspicuously at the seashore. Their shell consists of several (usually eight) separate, overlapping plates. Although this gives them the appearance of being segmented, their internal organs are not.

Until *Neopilina* (Fig. 1.2) was discovered in 1952, the class to which it belongs was thought to have been extinct for millions of years. This mollusk is of great interest because in addition to possessing all the typical molluscan features, it is segmented internally. In this latter respect, it is like the annelids and thus supports the widely held view that the mollusks and the annelids are closely related. The fact that mollusks also produce trochophore larvae provides additional evidence of their kinship with the

Many mollusks have trochophore larvae

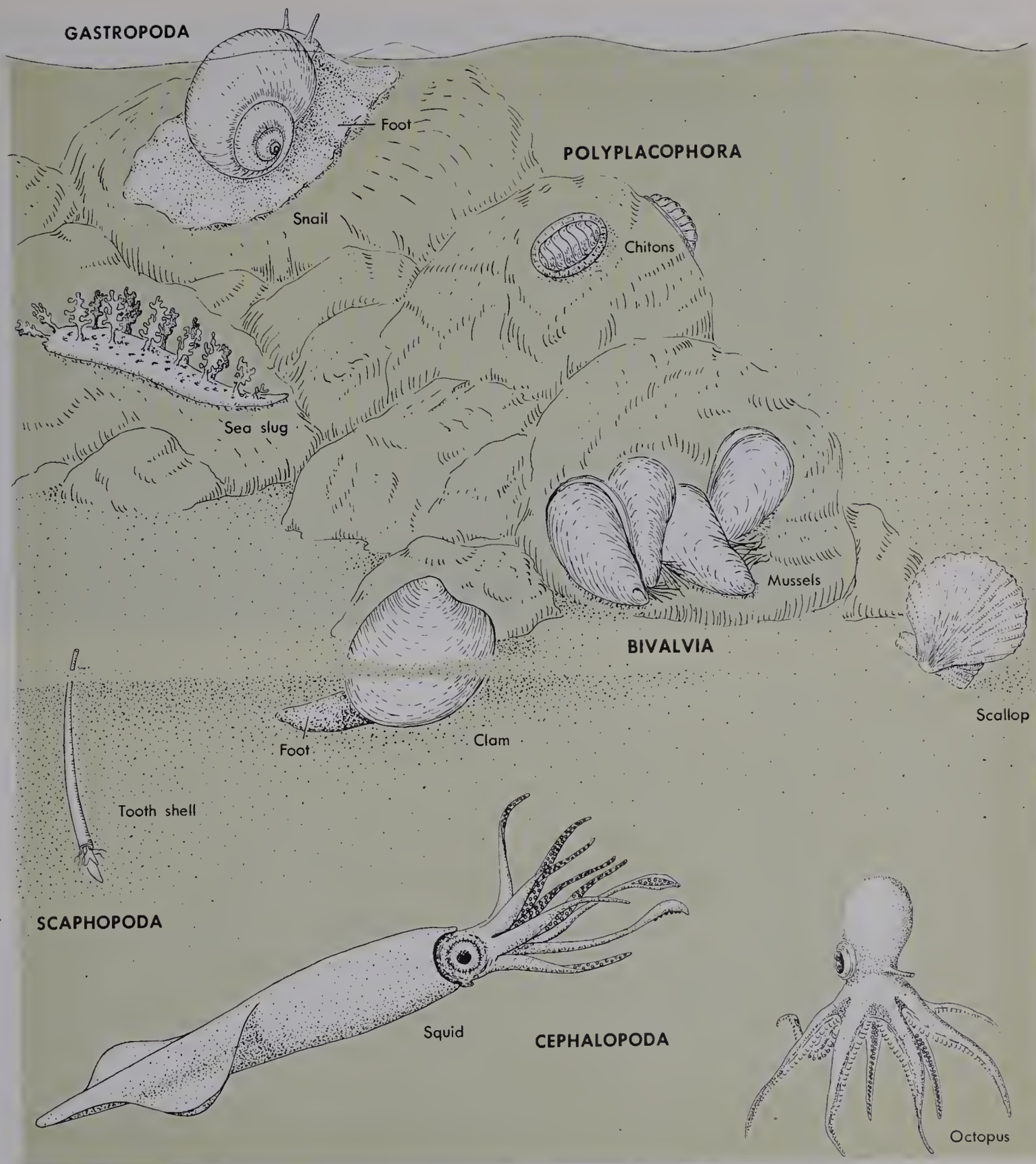


Fig. 36.11 Representative mollusks.



Fig. 36.12 The chambered nautilus. The shell has been cut to reveal its construction. (Courtesy of the American Museum of Natural History.)

annelids. However, the coelom—so characteristic of annelids—is greatly reduced in size in the mollusks. The main body cavity is the **hemocoel,** which is derived from the embryonic blastocoel.

Although the earliest mollusks were aquatic, the colonization of the land by plants opened up niches into which the snails and slugs were able to move. The protection of a shell, internal fertilization (sometimes embryonic development as well), and the modification of the mantle into a "lung" were all adaptations enabling them to shift from the aquatic to the terrestrial environment.

It is the evolutionary success of the snails and bivalves that makes the phylum one of the three that are dominant on the earth today. Of some 100,000 species, all but a few hundred belong to these two classes. It was not always so. The Ordovician and Silurian seas were well populated by the nautiloids, a group of shelled cephalopods whose only living descendants are three species of chambered nautilus (Fig. 36.12). Some of these forms possessed shells 15 ft long. In the Mesozoic era, the ammonites, another group of cephalopods, were the dominant invertebrates in the sea. Although now sharply reduced in numbers, the surviving cephalopods are still impressive. The 50-ft squid ranks as the largest invertebrate that ever lived.

36.9 THE ARTHROPODS (PHYLUM ARTHROPODA)

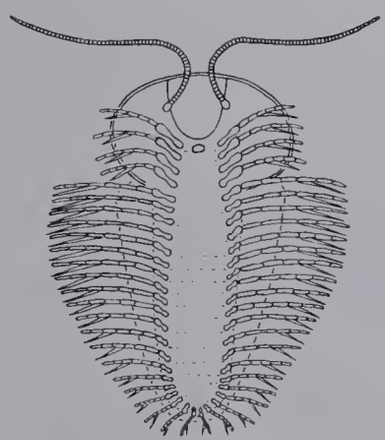
If number of species is any criterion, this is the major phylum on earth today. Over 765,000 different species of arthropods have been identified.

That is more than all the other species of living things—plant, animal, and protist—put together. Each year still more arthropod species are found, living in every conceivable habitat. Fresh water, salt water, soil, and practically the entire surface of the earth abound with them. They are almost the only animals to be found in the interior of Antarctica and on the snow- and rock-strewn slopes of our highest mountains.

Characteristics

All members of this phylum have a segmented body enclosed in a tough, jointed external skeleton made principally of a material called **chitin.** The symmetry is bilateral and is clearly marked by the pairs of jointed appendages arranged along either side of the midventral axis. In all living arthropods, the various appendages in a given species show considerable variety of structure and function. In addition to locomotion, they may aid in food-getting, in sensation, and as offensive and defensive weapons.

Unlike those of annelids, the segments of arthropods show considerable variation in structure from front to back. They are usually combined into three main regions: the **head, thorax,** and **abdomen.** Arthropods have a circulatory system which is considered "open" inasmuch as the blood (in contrast to that of the annelids) is not confined within blood vessels at all times. The main part of the nervous system of arthropods, like that of the annelids, runs along the ventral side of the organism.



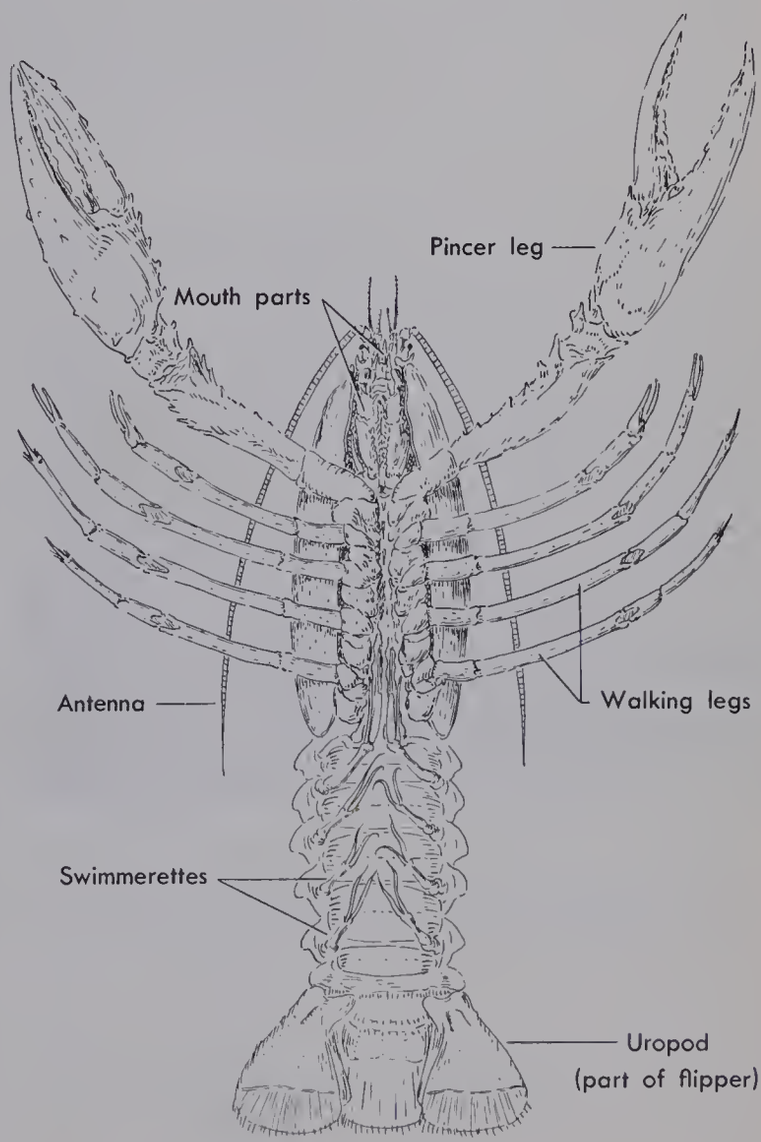


Fig. 36.13 A trilobite (above) and crayfish compared. The paired, jointed appendages of the crayfish show far more structural and functional specialization than do those of the trilobite.

Trilobites

Among the most abundant fossils of the Cambrian period are the **trilobites.** These early arthropods were segmented and had paired, jointed appendages and an exoskeleton just like those of their modern descendants. Although we suspect that the anterior appendages aided in food-getting while the posterior ones were used for locomotion, they were quite uniform in structure (Fig. 36.13). Aquatic **arachnids,** called eurypterids (Figs. 36.14 and 36.15), and primitive **crustaceans** also appear in the Cambrian. The eurypterids probably evolved from early trilobites, and the crustaceans must have been close relatives. Their chief structural advance over the trilobites was the development of a variety of modifications in their appendages. The modern crayfish with its flipper, swimmerettes, walking legs, pincer legs, specialized mouth parts, and antennae (Fig. 36.13) stands in sharp contrast to the trilobite. The structural diversity of these numerous crayfish appendages reflects the variety of functions carried out by them.

Crustacea

The crustaceans differ from other arthropods in having two pairs of antennae. In addition, the head and thorax regions are fused into a single **cephalothorax.** The class includes crayfish, lobsters, barnacles, crabs, and myriads of tiny shrimplike organisms. With the exception of the "pill bug," which lives under logs and stones, the crustaceans are aquatic, being found in both fresh water and salt water. All members of the class breathe by means of gills. They range in size from microscopic forms to lobsters and crabs weighing several pounds (Fig. 36.16).



Fig. 36.14 An artist's reconstruction of aquatic life of the Silurian period. The segmented animals are eurypterids, a now-extinct group of aquatic arachnids. (Courtesy of the Field Museum of Natural History.)



Fig. 36.15 Remains of a eurypterid. Courtesy of the American Museum of Natural History.

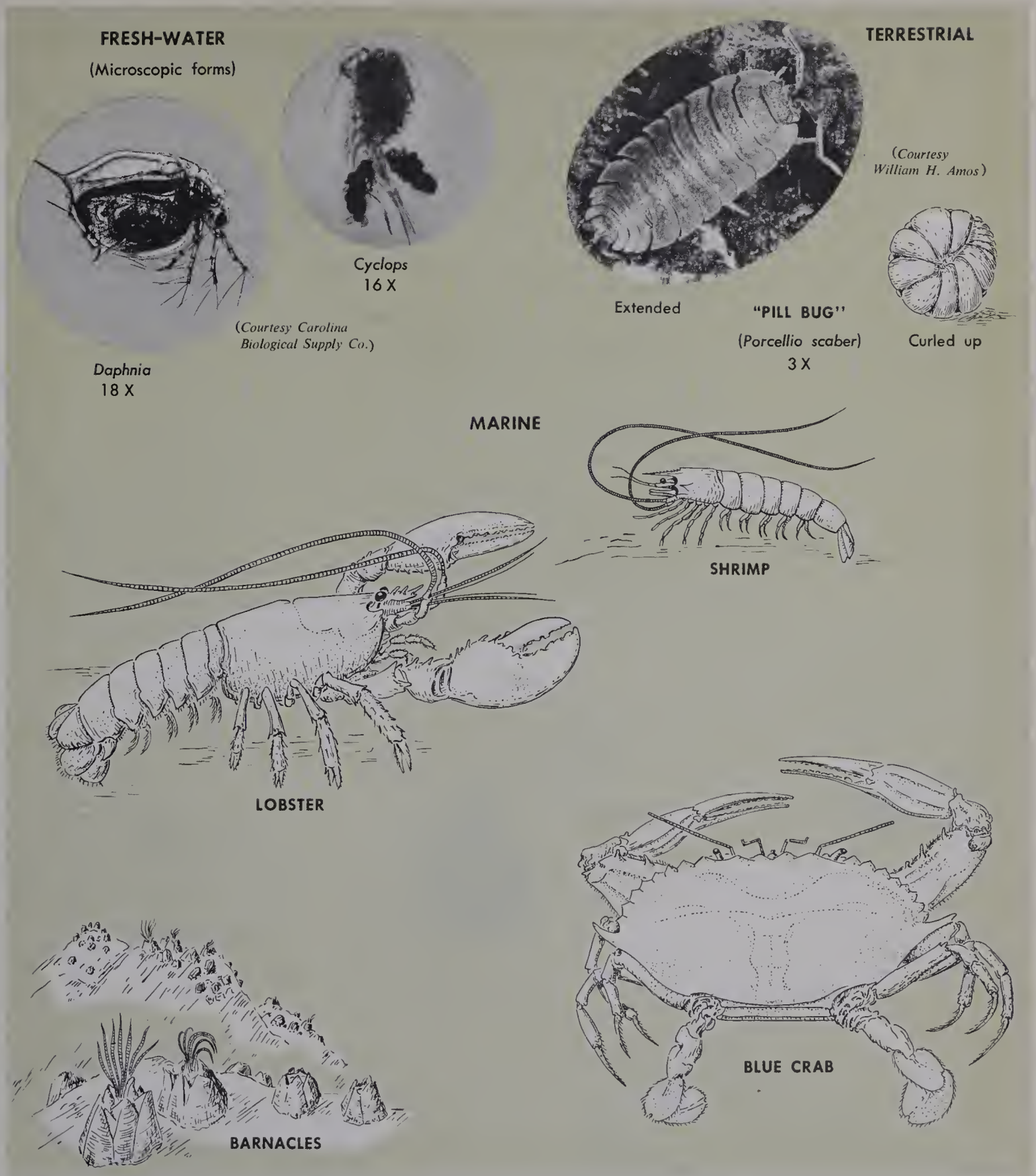


Fig. 36.16 Representative crustaceans. This class of arthropods has been most successful in the sea.

Arachnida

By Silurian times, terrestrial arachnids had evolved. The scorpions, mites, ticks, spiders, and daddy longlegs of today are their descendants (Fig. 36.17). The arachnids, like the crustaceans, have the head and thorax fused into a cephalothorax. Unlike the crustaceans, though, almost all the arachnids are terrestrial and breathe air. Locomotion is by four pairs of legs. Antennae are not present.

Although eurypterids as long as nine feet existed in the Silurian, they and the trilobites became extinct by the end of the Paleozoic era. The horseshoe crab *Limulus* is the only representative of the early aquatic arachnids to survive into modern times. It has done so with practically no evolutionary change since the genus first appeared in the Triassic period over 200 million years ago.

Diplopoda (millipedes) and Chilopoda (centipedes)

Millipedes and **centipedes** (Fig. 36.18) probably arose in the Devonian period, although clear fossil remains are found only as far back as Carboniferous times. **Insects,** however, were definitely established by the Devonian. This versatile, fast-reproducing, fast-evolving group has become the dominant class of arthropods in every habitat but salt water (where the crustaceans reign supreme). Over 700,000 currently living species of insects have been identified, which is over one-half of all the kinds of living things found upon the earth.

Insecta

The insect body is divided into three regions: head, thorax, and abdomen. The thorax itself consists of three distinct segments, each of which bears a pair of legs. Thus the insects are the six-legged creatures. Most of

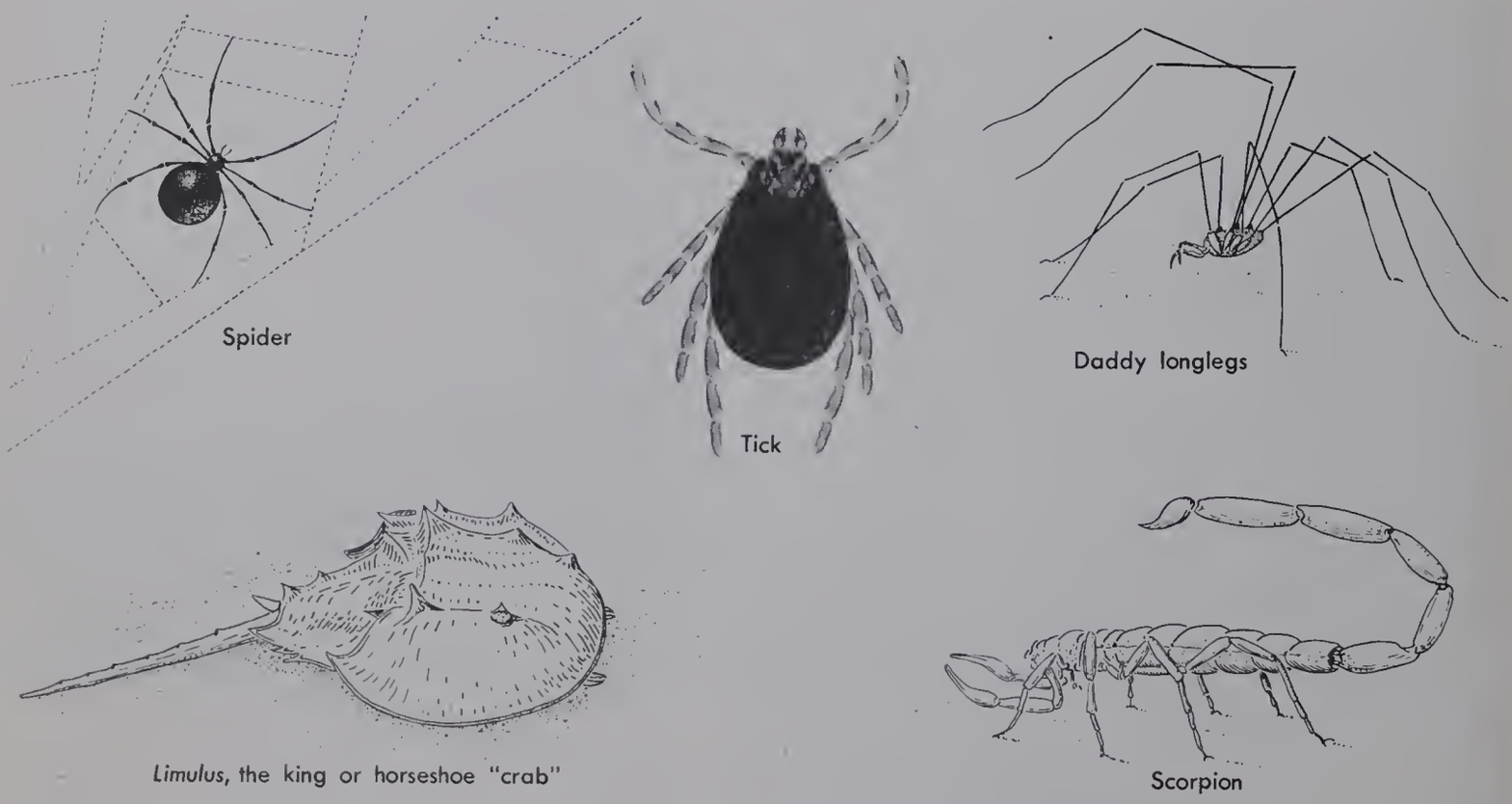


Fig. 36.17 Representative arachnids. With the exception of *Limulus,* the members of this class are land-dwellers. They have four pairs of legs.

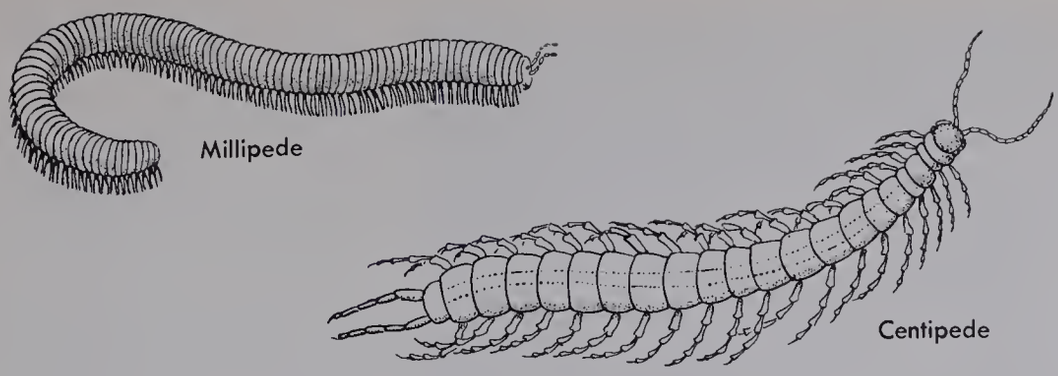


Fig. 36.18 Millipedes have two pairs of legs on each segment; the centipedes have only one pair per segment.

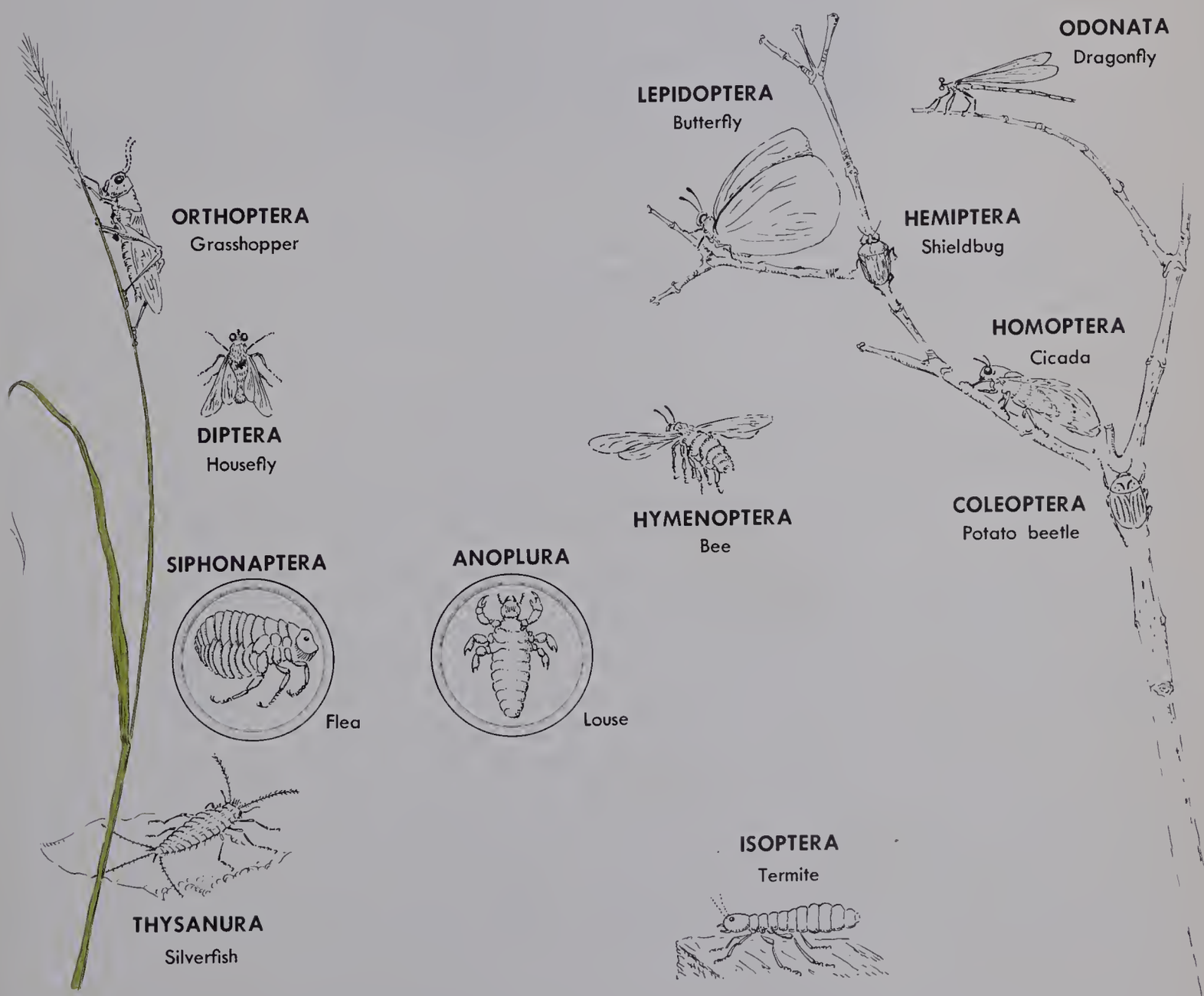


Fig. 36.19 Representatives of the major insect orders. All insects have six legs.

them, when adult, also have one or two pairs of wings on the thorax. They have a single pair of antennae mounted on the head.

With 700,000 species in one class, you can well imagine that a great deal of attention has been paid to the lesser taxonomic categories. About two dozen orders have been established on the basis of differences in metamorphosis, wing structure, and mouth parts. Figure 36.19 illustrates some of the major insect orders.

Of the several classes of arthropods, no other solved the problems of dry-land living so successfully. The exoskeleton is quite impervious to water and thus prevents fatal dehydration of the body when the surrounding air is dry. Gas exchange is accomplished by a system of tracheal tubes (see Section 12.5), which penetrate to every portion of the body. The paired, jointed, clawed appendages provide not only for locomotion but also for ingestion of food. Mandibles, maxillae, and labia are fashioned in remarkably diverse ways to produce sucking, biting, chewing, and rasping mouth parts.

Other adaptations to a terrestrial life included the development of wings for flying. (The insects were the first animals to use this medium for locomotion.) Internal fertilization and embryonic development within a waterproof egg solved the problem of bringing the gametes together and protecting the embryo without the aid of water. Finally, the ability to excrete their nitrogen-containing wastes as uric acid must be counted as one of the important evolutionary adaptations to a dry habitat. Not only does uric acid require very little water for its excretion, but the fact that what little is used is then reclaimed in the rectum enables these creatures to deposit wastes which are practically dry.

The efficiency of insect structure and function cannot be disputed. Both in terms of number of species and number of individuals, the insects earn a position as one of the most successful groups on earth. They are man's chief competitors for food. Except for the oceans, insects have adapted to practically every habitat available on the earth.

The fossil record tells us little about the evolutionary relationships of the millipedes, centipedes, and insects. However, a living animal, *Peripatus,* supports our view that they, too, evolved from a primitive segmented worm. *Peripatus* (Fig. 36.20) has internal segmentation, pairs of nephridia-like excretory organs in each segment, a cuticle, and a muscular body wall, all quite suggestive of the annelids. Furthermore, the excretory and sex organs are ciliated as they are in annelids, and the structure of the digestive tract, central nervous system, and legs is similar to that of annelids. On the other hand, the legs of *Peripatus* bear insectlike claws, and the circulatory system is open with the blood passing through an extensive hemocoel. Furthermore, these little creatures breathe air by means of tracheae, and the embryonic development of their eggs resembles that of arthropods.

Peripatus

Peripatus is certainly not the ancestor of the millipedes, centipedes, and insects. However, its curious anatomy suggests that it may be a relatively little-changed descendant of forms which also led to these arthropods. Fossil members of its phylum have been found side-by-side with

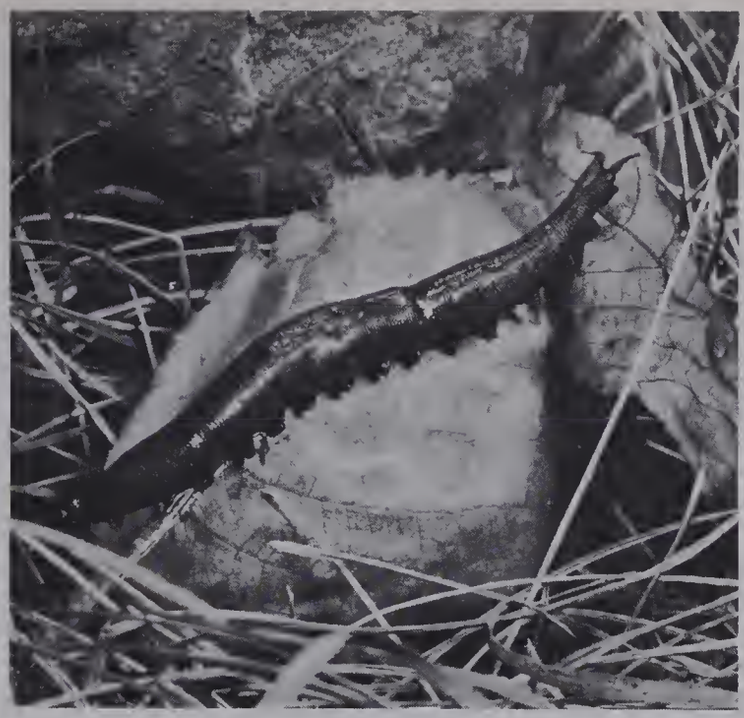


Fig. 36.20 *Peripatus.* This little animal has many features of both annelid worms and insects. It is found in a few tropical localities. (Courtesy of Ward's Natural Science Establishment, Inc.)

fossil trilobites, crustaceans, and annelids. The fact the *Peripatus* occurs in small, isolated parts of the jungles of Central and South America, Africa, Asia, and Australia indicates that it may represent the last-surviving remnants of a once-flourishing group.

36.10 THE ECHINODERMS AND CHORDATES

The echinoderms (phylum Echinodermata) consist today of about 6000 species, all of which live in salt water. The phylum is made up of five classes, the scientific names and typical representatives of which are given in Figure 36.21.

The three diagnostic features of the chordates

The chordates (phylum Chordata) include more than 44,000 species. All of them have bilateral symmetry, some degree of segmentation, and an internal skeleton. What sets them apart from all the other animals, however, is three unique features (Fig. 36.22): (1) All chordates possess a flexible, rodlike structure, the **notochord,** at some stage of their development. This is located dorsal to the digestive tract and provides internal support for the body. In most chordates, it is replaced by a vertebral column or backbone long before maturity is reached. (2) At some stage in development, all chordates possess pairs of **gill pouches.** These are lateral outpocketings of the throat. They are matched on the exterior by paired grooves. In aquatic chordates, the gill pouches break through to the exterior grooves, forming **gill slits.** These provide an exit for water that is taken in through the mouth and passed over the gills. In the land chordates, the gill pouches do not break through but become greatly modified during the later course of development. (3) All Chordates possess a hollow nerve cord that develops on the dorsal side of the body above the notochord. At its anterior end, it becomes enlarged to form the brain.

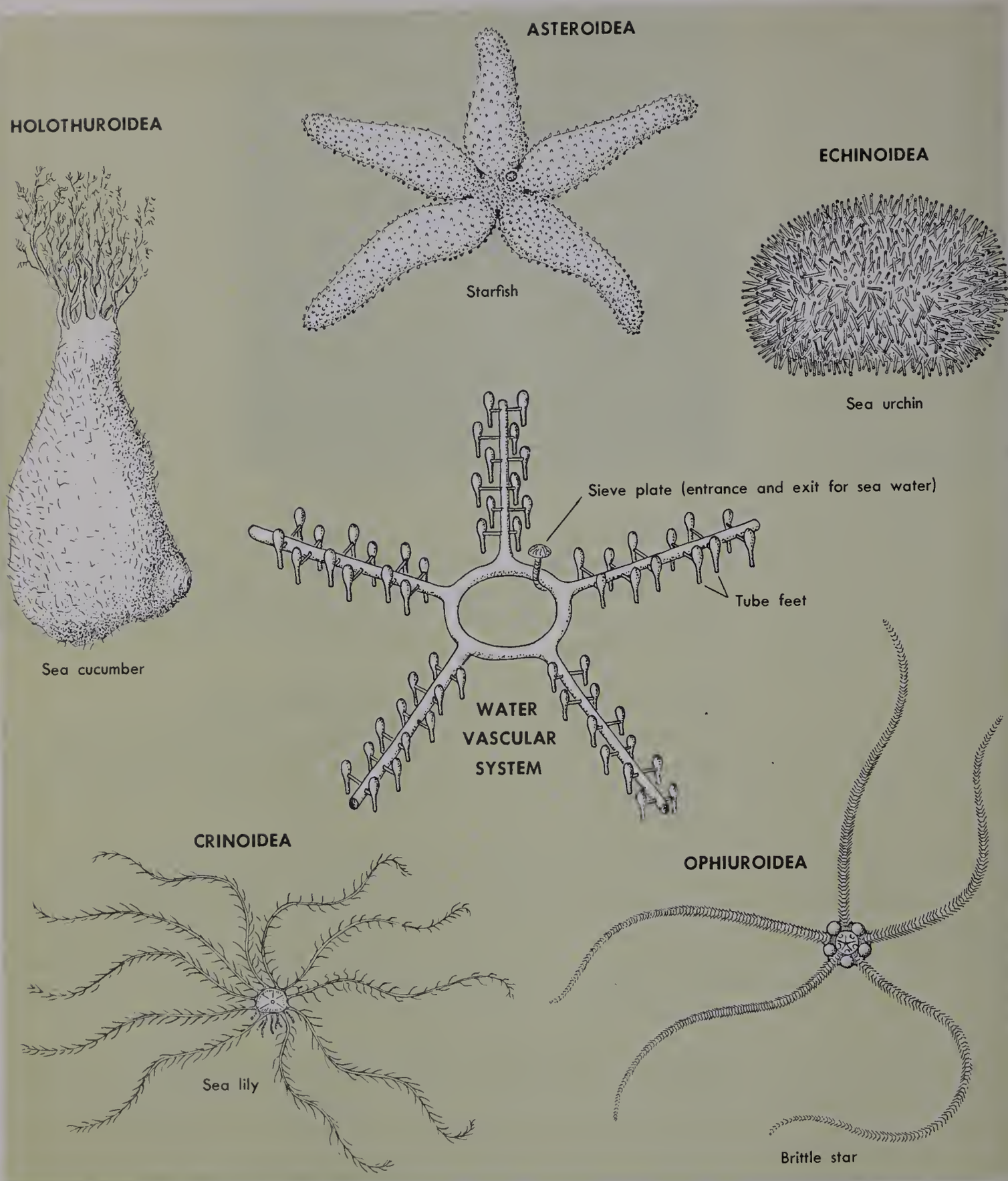
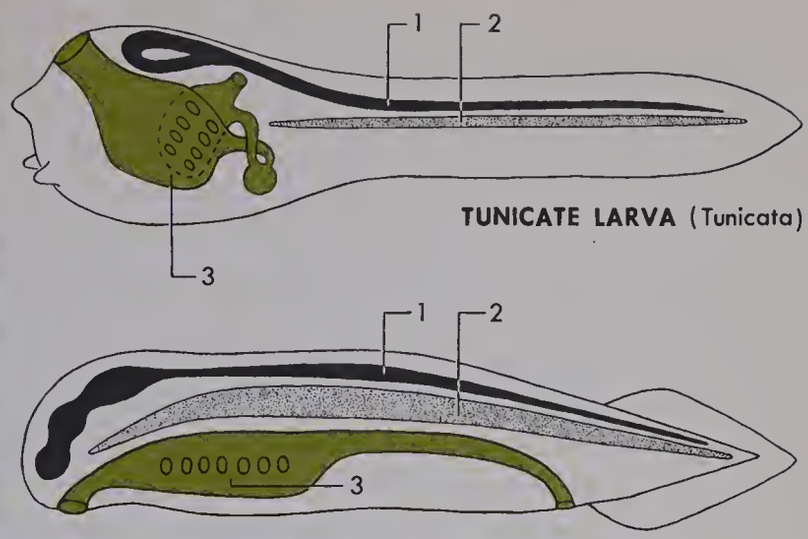


Fig. 36.21 Representative echinoderms. All members of the phylum live in salt water, have a spiny skin and radial symmetry (as adults), and move slowly with the aid of their tube feet. Extension and retraction of the tube feet is accomplished by the movement of sea water in and out of the water vascular system.

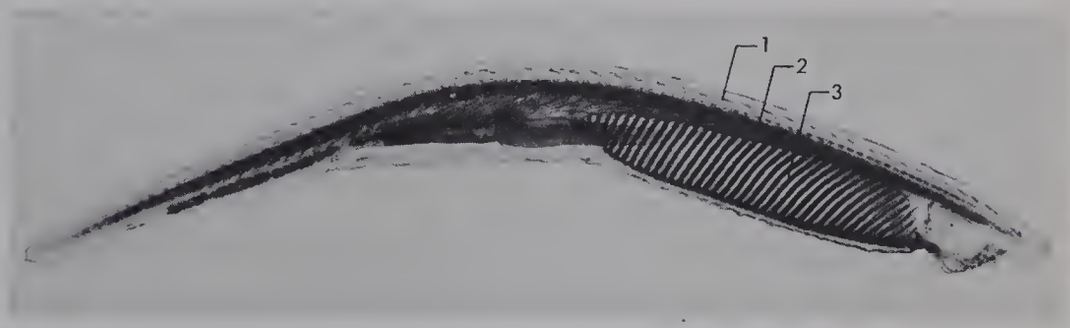


ADULT TUNICATE (*Halocynthia*)
(*Courtesy Ralph Buchsbaum*)



TUNICATE LARVA (Tunicata)

LARVA OF A JAWLESS FISH (Vertebrata)



1. Dorsal nerve cord
2. Notochord
3. Gill slits

AMPHIOXUS (Cephalochordata)
(*Courtesy General Biological Supply House, Inc.*)

Fig. 36.22 Three chordates. Although the adult forms of these three animals differ strikingly, at some time each shows the three basic chordate characteristics listed.

The vast majority of chordates are vertebrates and will be discussed in the next chapter. A few chordates, however, do not develop backbones, and these may provide clues to the origins of this remarkably successful group of animals.

Echinoderms and chordates share a number of features between themselves which do not occur in the other animal phyla. This is particularly apparent in their embryonic development.

Contrasts between the echinoderm superphylum and the annelid superphylum

As we saw in Fig. 22.1, the cleavage furrows in chordate eggs occur in regular planes either perpendicular or parallel to one another. This is also true of echinoderm eggs. However, in all the other animals we have discussed (with the possible exception of the arthropods, whose method of cleavage is unusual), the planes of cleavage are progressively tipped with respect to one another so that a *spiral cleavage* results.

The cells produced by the first cleavage or two in echinoderm and chordate eggs are fully capable of developing into a complete embryo if isolated from each other. For this reason identical twins can and do occur in both groups. On the other hand, cleavage in the invertebrates which we

have studied up to this point results in cells that have lost some of their potentiality. Identical twins do not occur.

In the echinoderms and chordates, gastrulation begins at that portion of the embryo which will become the anus of the adult (see Section 22.3). However, in the other phyla with a complete digestive tract, it begins at the end which ultimately becomes the mouth. Both groups possess a coelom, but the mechanism of its formation is quite different. In annelids, arthropods, and mollusks, the coelom arises by a splitting of a mass of mesodermal cells which have their origin within the embryo. In echinoderms and chordates, the coelom develops within a sheet of cells which pushes in from the exterior during gastrulation. One other distinction between these two groups which deserves mention is a chemical one. As you learned in Chapter 28, the compound creatine phosphate serves as a reservoir of high-energy phosphate in the muscles of man. It also performs the same function in all other vertebrates and in many of the invertebrate chordates (e.g. *Amphioxus*—Fig. 36.22) and in some echinoderms (e.g. brittle stars). Most of the other invertebrates that have been examined have a related substance, arginine phosphate, which we assume performs a similar function for them. The sea urchins, acorn worms (Fig. 36.23), and tunicates (Fig. 36.22) have one or the other or both of these substances.

The occurrence of so many sharp contrasts between the echinoderms and chordates on the one hand and most of the remaining animals on the other has led some taxonomists to establish two superphyla: the echinoderm superphylum (to which we belong) and the annelid superphylum. It has also made it quite clear that we cannot expect to find clues



Fig. 36.23 An acorn worm (Hemichordata). This marine animal has both echinoderm and chordate features. (Courtesy of William H. Amos.)

to the evolutionary origins of man by examining such advanced and distantly related forms as mollusks and arthropods. How, then, can we account for the origin of vertebrates and, thus, of ourselves? Fossil echinoderms were present in the Cambrian so we know that the echinoderm superphylum was established before that time. We do not know from which more primitive group it evolved. One that has been suggested as possibly ancestral to the echinoderms and chordates is the Nemertina. This phylum, which has not been discussed before, contains a small group of flattened, ciliated worms that are generally thought to be closely related to the flatworms. But one important way in which they differ from the flatworms is that gastrulation produces a one-way digestive tract with both mouth and anus. In some nemertines, however, the site of gastrulation (the blastopore) becomes neither the mouth nor the anus. Instead it becomes sealed over in later embryonic development, and mouth and anus formation occur on opposite sides of it. Perhaps these worms are descendants of a group in which the site of gastrulation was in transition from the mouth end of the archenteron (annelid superphylum) to the anus end (echinoderm superphylum). The hypothesis is supported by the discovery of some two dozen structures in the nemertines which seem to be homologous to structures found in one of the most primitive vertebrates, the hagfish.

Do the nemertine worms bridge the two superphyla?

Assuming that the echinoderm superphylum is descended from nemertine ancestors, we must now attempt to unravel the evolutionary relationships within the superphylum. All of the chordates are bilaterally symmetrical. The echinoderms, on the other hand, are radially symmetrical. Their radial symmetry should not fool us, though, for the larvae of the echinoderms are bilaterally symmetrical. Adhering to our conviction that embryonic features are the more primitive ones, we conclude that the radial symmetry of adult echinoderms is a secondary adaptation to a sessile or, at most, sluggish way of life. (Remember the cnidarians.) The fossil record tells us that the earliest echinoderms were sessile animals attached by means of a stalk. Some of the modern descendants of these early echinoderms (the sea lilies) are still sessile, and all of them at least pass through a stalked stage.

Echinoderm traits

The echinoderms have been moderately successful in their rather limited habitat of the oceans. From the earliest stalked members of the phylum arose the other classes: the sea urchins, brittle stars, starfish, and sea cucumbers (Fig. 36.21). The abundance of these organisms, their internal skeleton, and their marine habitat have resulted in a remarkably complete fossil record of their evolution.

The invertebrate chordates

The position of the acorn worms (Fig. 36.23) in this story is puzzling. A notochordlike structure, gill slits, and what has been claimed to be a dorsal central nervous system have led to their being classed as chordates by some biologists. Their affinity with the echinoderms is undisputed, however. The larva of the acorn worm is practically indistinguishable from the larva of the sea cucumber. Acorn worms have both creatine phosphate and arginine phosphate as do the sea urchins. Finally, comparative

serology clinches the argument. Antibodies developed in response to acorn worm proteins also react strongly with sea cucumber proteins. No wonder, then, that these animals with *both* chordate and echinoderm features have been placed in a phylum (Hemichordata) of their own.

Despite the superficial resemblance of *Amphioxus* to fishes (Fig. 36.22), it is to the tunicates (Subphylum Tunicata) that we look for the origin of the vertebrates. Actually, examination of an adult tunicate such as the sea peach, *Halocynthia,* could not reveal a more unlikely-looking candidate for vertebrate ancestry. This sessile, filter-feeding organism with its "tunic" containing cellulose has no notochord and only a rudimentary nervous system. However, the larvae produced by these organisms are strikingly different in form. They are free-swimming, bilaterally symmetrical "tadpoles" with gill slits, a dorsal tubular central nervous system, and a tail through which runs a notochord. In fact, if one tries to visualize what a primitive, idealized chordate should have looked like, he arrives at an organism not much different from a tunicate larva.

One difficulty remains. The fossil evidence suggests that vertebrates arose in fresh water while the tunicates are exclusively marine. The tunicates do, however, live close to shore and their larvae could have entered the mouths of rivers. Furthermore, the steady one-way current of river water may well have altered selection pressure in favor of an active, bilaterally symmetrical form. Thus, the ancestral tunicate larva may have abandoned metamorphosis to the adult and provided the start of vertebrate evolution. The abandonment of metamorphosis is not unknown elsewhere. Remember the axolotl (Fig 25.5), which reaches sexual maturity as a gill-breathing larva. Here, too, the environment (lack of iodine) has selected for the retention of juvenile traits.

EXERCISES AND PROBLEMS

1. If someone brought a radially symmetrical animal to you, what conclusions might you draw about (a) its habitat, (b) its power of locomotion, (c) its feeding habits?
2. For each of the following, tell whether it belongs to the annelid or echinoderm superphylum: (a) trochophore larva, (b) tunicate, (c) identical twins, (d) squid, (e) sea cucumber.
3. The largest vertebrate and the largest invertebrate live in the ocean. Can you think of an explanation for this?
4. Hemoglobins consist of a protein to which is attached the prosthetic group, heme. Hemoglobins are found in vertebrates, *certain* mollusks, nemertines, annelid worms, insects, crustaceans, nematodes, echinoderms, and even in leguminous plants. Does their presence in these forms indicate close kinship? If not, can you account for the repeated independent evolution of hemoglobins?

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37 THE EVOLUTION OF THE VERTEBRATES

The Jawless Fishes (Agnatha)

The Placoderms

The Cartilaginous Fishes

The Bony Fishes

The Amphibians

The Reptiles

The Birds

Continental Drift

The Evolution of the Mammals

The Evolution of the Primates

The Evolution of Man

37.1 THE JAWLESS FISHES (AGNATHA)

The ostracoderms had neither jaws nor fins

The first vertebrates to appear in the fossil record are jawless fishes, the **ostracoderms.** Some appear in Ordovician rocks, although they become far more abundant in the Silurian. These creatures were relatively small (6 to 12 inches) flattened fishes (Fig. 37.1), which probably lived by sucking up organic debris from the bottom of the streams they inhabited. Gas exchange occurred at the pairs of internal gills, each gill supported by a bony arch. Water entered the mouth, passed over the gills and left through a series of gill pouches which opened out at the surface. There were no fins, the fish swimming by means of undulating movements.

The body was encased in an armor of bony plates. This may have provided some protection against the large eurypterids which shared the same habitat. It may also have reduced the inflow of water in the hypotonic environment. The gills had to be exposed to the water, however, so some continual inflow was unavoidable. The solution to the problem was to use the pressure created by the contraction of the heart to pump water back out of the body. The ostracoderm probably had inherited nephridialike excretory tubules from its invertebrate ancestors. The development of a glomerulus near the opening (the nephrostome) of each permitted the transfer of fluid (nephric filtrate) from the blood to the tubule and thence to the exterior. Of course, valuable materials (e.g. salts) would also be lost by such a mechanism so we must assume that reabsorption of such materials occurred in the tubules. The presence of a capillary network draining the glomeruli provided the increased blood supply necessary for increased tubular reabsorption. Thus evolved the first kidneys.

They were not primarily organs for excretion but for maintaining water balance in a hypotonic environment. Nitrogenous wastes (chiefly ammonia) were probably excreted at the gills. This is certainly true of the modern fresh-water fishes. This first kidney did, however, establish a pattern which was to be adapted to the excretory and water-balance needs of each of the later vertebrate classes.

Modern jawless fishes

The only jawless fishes left today are the lamprey (Fig. 37.2) and the hagfish. These creatures are still the most primitive of the vertebrates. Besides lacking jaws, they have no paired fins. The notochord persists throughout the life of the organism. It is never completely replaced by the skeleton, which is made of cartilage. There are no scales on the body.

Despite its isolated position in the world of the vertebrates, the sea lamprey has proved to be a great nuisance to man. It feeds by attaching itself with its sucking mouth to the bodies of true fishes and sucking their tissue fluids. With the construction of the Welland Canal around Niagara Falls, the sea lamprey has been able to move into the Great Lakes from its former marine habitat. In a remarkably short time, it has virtually wiped out the large lake trout fishing industry that once existed. The recent discovery of a chemical that selectively kills young lampreys while sparing other organisms gives promise that the numbers of this pest may eventually be reduced and the fish population of the Great Lakes restored.

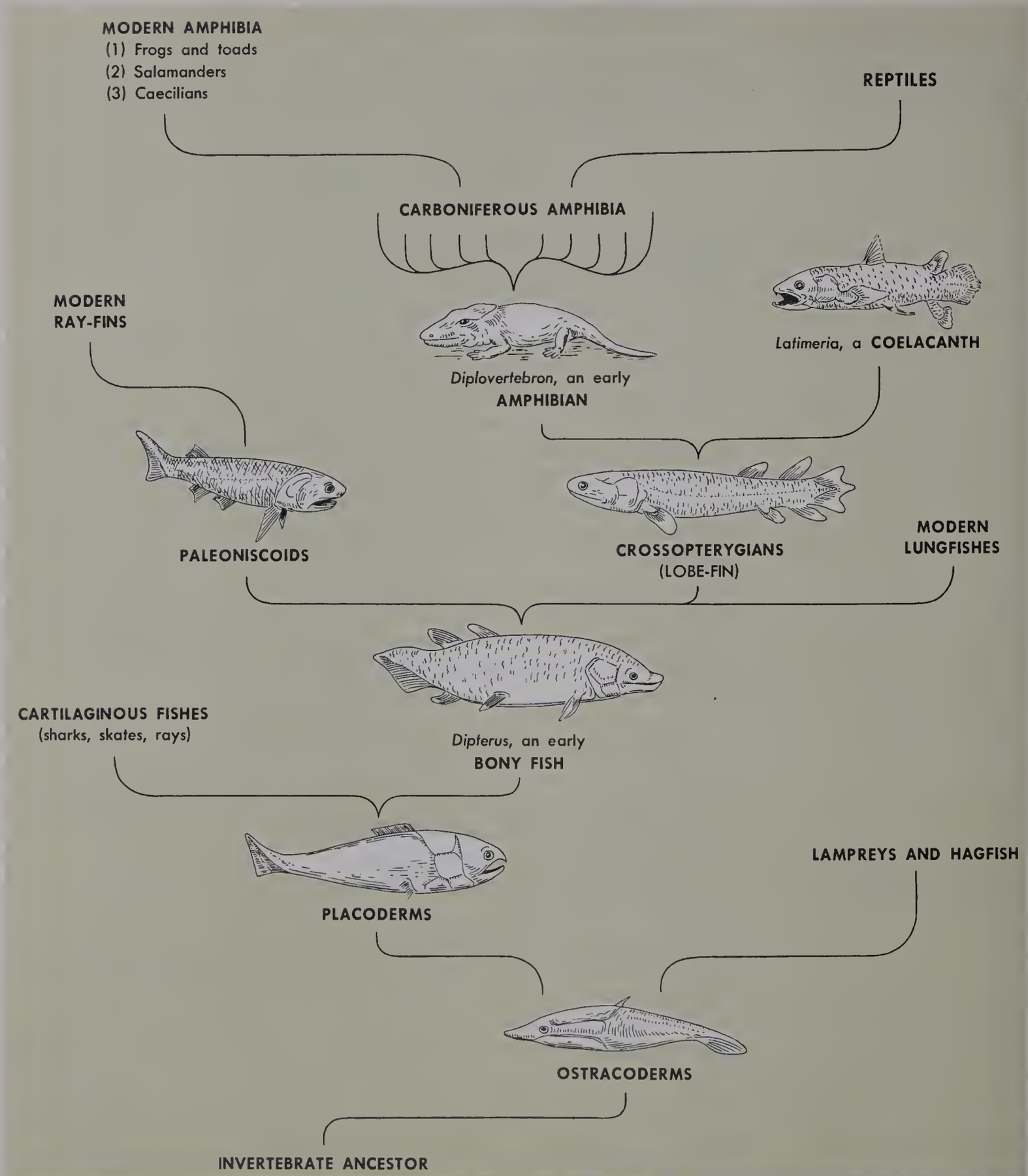


Fig. 37.1 The evolution of the fishes and amphibians.



Fig. 37.2 The sea lamprey. Note the presence of gill slits and the absence of paired fins. (Courtesy of the Carolina Biological Supply Co.)

37.2 THE PLACODERMS

Placoderms had jaws and fins

A second group of armored fishes appeared early in the Devonian. These placoderms (Fig. 37.1) differed from their agnath ancestors in two fundamental respects. They had jaws and they had paired fins. The former enabled them to prey actively on smaller animals; the latter aided locomotion by stabilizing the fish in the water. The resulting shift away from bottom feeding was also reflected in the development of a cylindrical body in contrast to the flattened body of the agnaths.

The fossil record reveals an extensive adaptive radiation of these fishes during the Devonian. Most of them later became extinct, but some produced lines of descent which led to the two major classes of modern fishes, the cartilaginous and bony fishes, the Chondrichthyes and Osteichthyes respectively. (Actually *bony* fish is not a uniquely descriptive name for the latter because their placoderm and agnath ancestors also had skeletons of bone.)

The Devonian was marked by periods when many lakes and streams dried up or became much smaller and warmer. This environmental change must have imposed a rigorous selection pressure on the freshwater Devonian fishes. Two solutions to the problem lay open to these animals, and both were taken. On the one hand, they could return to the ocean. On the other, the development of lungs for air-breathing would enable them to survive temporary periods of diminished water supply.

37.3 THE CARTILAGINOUS FISHES

The class Chondrichthyes includes the sharks, skates, and rays

Retreat to the oceans was the method of escape taken by the first cartilaginous fishes. These fishes (the earliest were sharks little different from our modern forms) get their name from the fact that their skeleton is made of cartilage, not bone. Like the placoderms, the sharks have jaws. The jaw "bones" developed from the first two pairs of gill arches (Fig. 37.3).

It is worth noting that in so doing, one pair of gill slits was no longer required. However, the opening still persists in some modern fishes. It is called the spiracle.

When the first cartilaginous fishes returned to the sea, they were exchanging a hypotonic environment (fresh water) for a hypertonic one. Instead of being faced with the problem of getting rid of excess water, they had to develop a method of conserving body water against the dehydrating effects of the sea. As you know, they accomplished this by converting their nitrogenous wastes into urea and allowing its concentration to build up in the blood until the blood was isotonic to the sea water. Today this requires a concentration of 2.5% which far exceeds that found in other vertebrates (0.02%). Because the ability to reach osmotic balance with the sea by this method develops late in the embryology of these animals, the eggs cannot simply be deposited unprotected in the water.

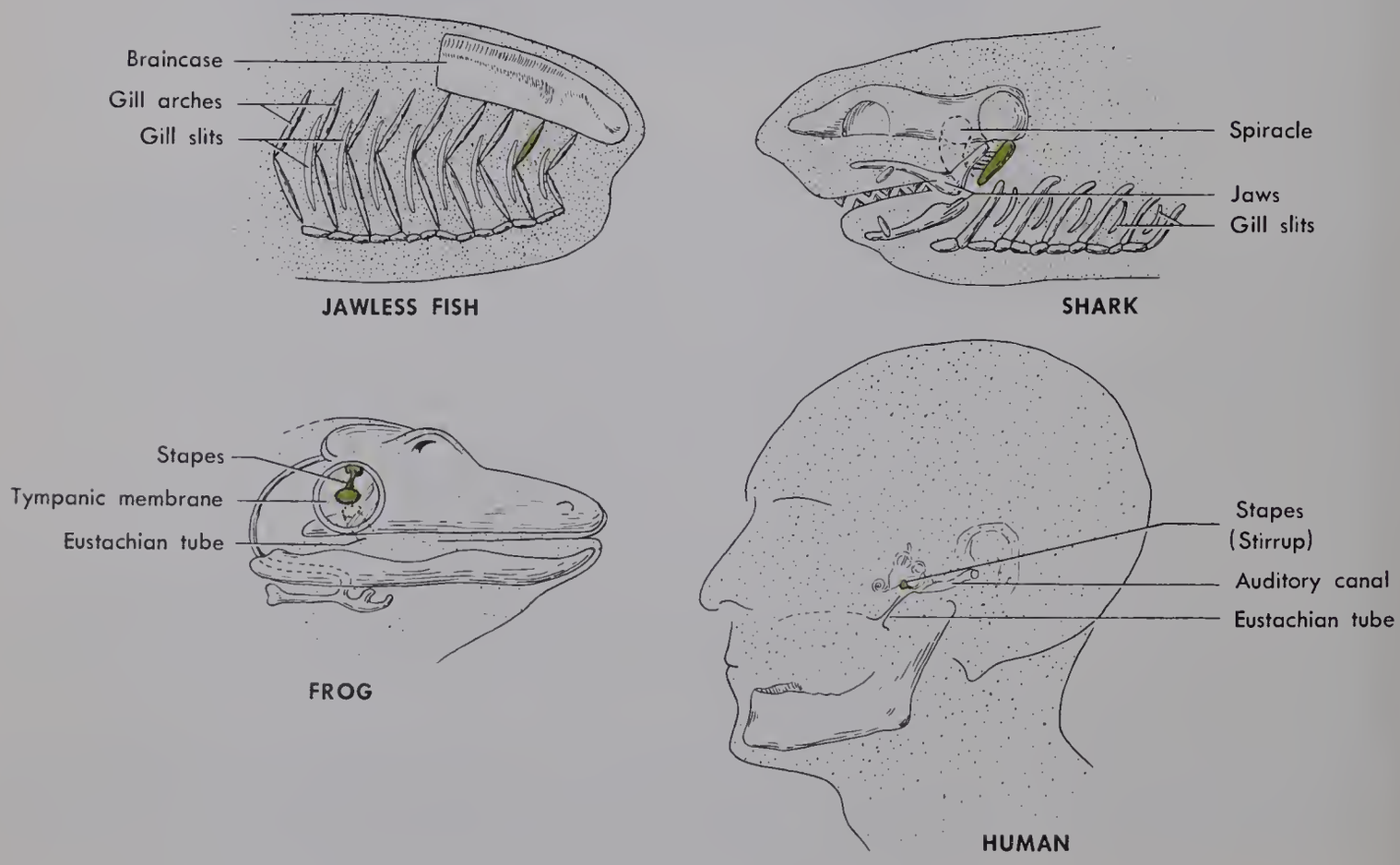


Fig. 37.3 Evolutionary transformation of the first gill slit and second (hyoid) gill arch of the jawless fishes. The bones homologous to the upper portion of the hyoid are shown in color.

One solution to this problem is to deposit the egg, surrounded by a suitable fluid medium, in an impervious case in which embryonic development can occur. A second solution is simply to retain the eggs, and the embryos into which they develop, within the mother's body until they are capable of coping with the sea-water environment. Both these solutions require that internal fertilization take place, and the cartilaginous fishes were the first vertebrates to develop this. The pelvic fins of the male are specially modified for depositing sperm in the reproductory tract of the female.

37.4 THE BONY FISHES

The first bony fishes had lungs

The so-called bony fishes (Class Osteichthyes) employed the second solution to the problem of periodic droughts. They developed a pair of pouched outgrowths from the pharynx which served as primitive lungs. They were inflated by air taken in through the mouth. The bodies of these fishes were covered with scales, the only vestige of their ancestors' armor being the bones of the cranium.

These fishes quickly (still in the Devonian) diverged into three distinct groups, the paleoniscoids, the lungfishes, and the crossopterygians.

All but a handful of modern bony fishes are descended from the Devonian ray-fins

The **paleoniscoids** were distinguished by the presence of ray-fins (fins in which neither muscle nor bone was present) and the fact that ventilation of the lungs continued through the mouth. Many of them migrated to the sea during the late Paleozoic and the Mesozoic eras. In a stable aquatic environment, lungs were not necessary, and they were transformed into a swim bladder by means of which the fish could alter its buoyancy in the water. All of our modern commercial fish, both marine and fresh-water (e.g. salmon, tuna, mackerel, trout, and bass) are descendants of this group.

Modern lungfishes have a number of adaptations that enable them to survive periodic draught

The **lungfishes** developed a significant innovation not possessed by their ancestors. Their nostrils, which in the first Osteichthyes had opened only to the outside and were used for smelling (as in all modern paleoniscoid descendants), developed internal openings to the mouth cavity. This made it possible to breathe air with the mouth closed. Once a rather successful group, the lungfishes of today are found in a few restricted localities of Africa, Australia, and South America, where their lungs still enable them to survive periodic droughts (Fig. 37.4). The widely scattered distribution of these fishes indicates that they are isolated remnants of a once-widespread group.

Judging from presentday lungfishes, two other significant adaptations evolved in this group. One was the development of two atria and a partial septum in the ventricle of the heart. This permitted at least a partial separation of oxygenated blood returning from the lung(s) and deoxygenated blood returning from the rest of the body and thus provided a marked improvement in circulatory efficiency (see Section 13.6). The second adaptation was the development of the enzyme system necessary to convert ammonia into the less toxic urea. This is particularly well developed in the African and South American species. While in the water, these



Fig. 37.4 The African lungfish surfaces from time to time to fill its lungs with air. Its gills are vestigial. (Courtesy of the American Museum of Natural History.)

fishes excrete their waste nitrogen as ammonia just as all the ray-fins do. In times of drought, however, these animals burrow into the mud and switch over to urea production.

The **crossopterygians** also had internal nostrils through which the lungs could be inflated. In addition, their pectoral and pelvic fins were lobed; that is, they were fleshy and were supported by bones. A glance at Fig. 37.5 shows that the pattern of bones (a bony girdle articulating with a single bone which, in turn, articulates with two others, etc.) is the one we find in all four-legged vertebrates. Each of our arm and leg bones is homologous to a bone in the crossopterygian pectoral and pelvic fins, respectively.

The lobe-fin fishes were the ancestors of the amphibians

Fossil remains tell us that the ancient lungfishes, like some of their modern descendants, burrowed in the mud and became dormant during periods of drought. The crossopterygians with their lobe-fins were able to attack the problem more aggressively. Their lobe-fins enabled them to waddle from one shrinking pool to another in search of better conditions. The combination of lungs and lobe-fins thus provided the necessary equipment to enter a new, uncrowded habitat, the land. One group of crossopterygian descendants did just that, in a rather tentative manner. These were the amphibians. Another group turned instead, as so many other Devonian fishes had done, to the sea. These, the coelacanths, were quite successful for a time in their new habitat but became extinct by the end of the Mesozoic era, some 70 million years ago. Or so everyone believed until 1938. In that year a living coelacanth *(Latimeria)* was pulled up from the ocean depths off the East Coast of Africa. Since then more than two dozen specimens have been caught. These "living fossils" still have paired lungs like those of their ancestors, but they are no longer functional.

The Devonian has been called the "Age of Fishes." This is surely appropriate in view of the extraordinary adaptive radiation which took place in the group during that period. Both fresh water and salt water became well populated by them. Toward the end of the Devonian, however, a new group of vertebrates appeared. These were the amphibians, the first of the four-legged vertebrates or **Tetrapods.**

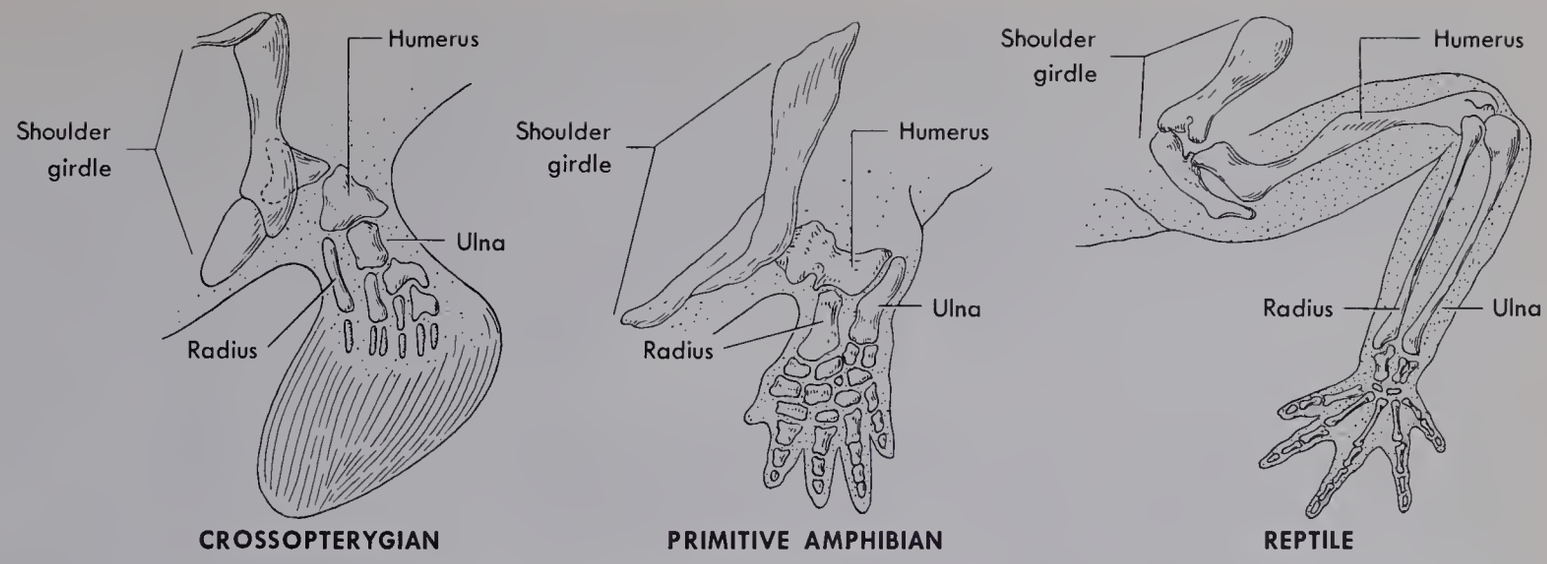


Fig. 37.5 Evolutionary development of the tetrapod forelimb from the pectoral fin of a crossopterygian.

37.5 THE AMPHIBIANS

The amphibians were the vertebrate pioneers of the land. Their lungs and bony limbs, inherited from their crossopterygian ancestors, provided a means of locomotion and a means of breathing air. A second atrium in the heart permitted oxgenated blood to be returned directly to it for pumping to the body under full pressure. While some mixing of oxygenated and deoxygenated blood occurred in the single ventricle, the three-chambered heart must have provided a substantial increase in circulatory efficiency and thus ability to cope with the more changeable, demanding land environment.

Evolution of the ear

On land, the ability to detect sound is of great importance, and the amphibians developed simple ears out of structures inherited from their ancestors. The spiracle (Fig. 37.3) was covered with a membrane that served as an eardrum and a no-longer-used jawbone (itself originally derived from an agnath gill arch) served to transmit vibrations from this membrane to the inner ear. The innermost ossicle (the stirrup) of our middle ear is homologous to this bone.

The amphibian kidney

As their name implies, the amphibians were only semiterrestrial. They had to return to water in order to lay eggs, and their modern descendants, at least, cannot withstand long exposure to dry air. The periodic shift from water to land and back again imposes additional problems in maintaining water balance and excreting nitrogenous wastes. In the water, the continual inflow must be pumped out of the glomeruli, as in the fresh-water fishes. On land, water must be conserved, and the amphibian accomplishes this by reducing the blood supply to the glomeruli and, hence, the filtration rate. Of course, this also diminishes the blood flow from the glomeruli to the tubules. Tubular function must be maintained, however, and increased activity of the auxiliary renal portal system (Fig. 15.10) permits this.

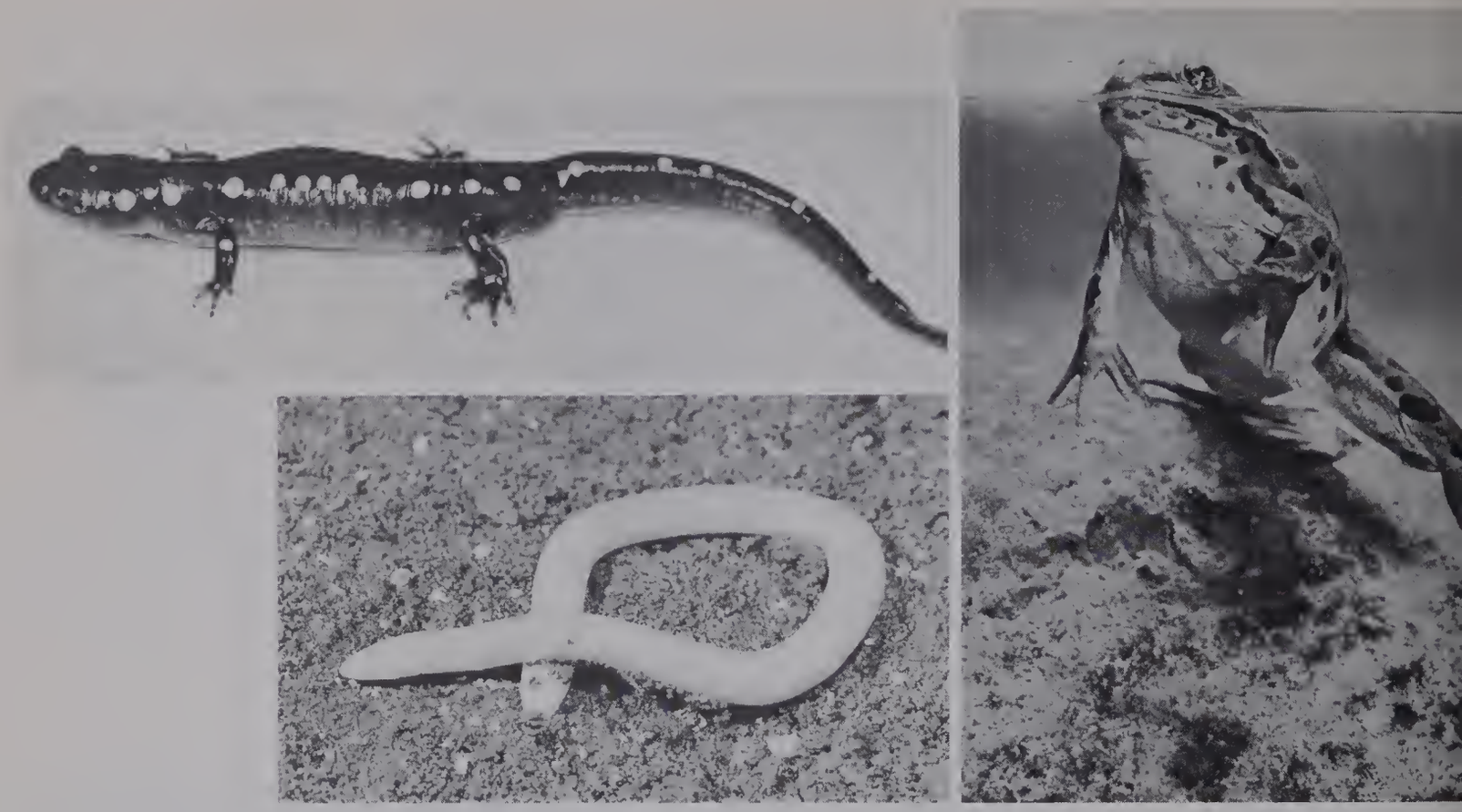


Fig. 37.6 Representative amphibians. Upper left: The tiger salamander is a common inhabitant of moist woods. (Courtesy of the Carolina Biological Supply Co.) Right: The leopard frog is a favorite laboratory animal. (Courtesy of Ward's Natural Science Establishment, Inc.) Lower left: The wormlike caecilians are found in the tropics. (Courtesy of New York Zoological Society.)

The earliest amphibians were large enough by modern standards *(Diplovertebron,* Fig. 37.1, reached a length of about 2 ft), but some of the later forms were truly of impressive size. Some fossil specimens are 8 ft long. These amphibians flourished during Carboniferous times. Vast swamps covered the earth, plant life was abundant, and there were plenty of insects on which the amphibians could feed. This period is often referred to as the Age of Amphibia.

It was followed by a period (the Permian) when the earth became colder and dryer. A decline in the fortunes of the amphibians set in that has continued to this day. Only three orders remain today: frogs and toads, salamanders, and the limbless, wormlike caecilians (Fig. 37.6). Lacking a waterproof skin and waterproof eggs, these creatures have never fully adpated to the conditions of land life.

37.6 THE REPTILES

The first truly terrestrial animals were the reptiles. These evolved from amphibians during Carboniferous times. With the arrival of the Permian, they were able to cope with the new conditions better than the amphibians.



Fig. 37.7 American chameleon emerging from egg. (Courtesy of the Carolina Biological Supply Co.)

The stem reptiles evolved a shelled egg

The chief advance the earliest reptiles (the "stem" reptiles or **cotylosaurs**) showed over the amphibians was the development of a shelled, yolk-filled egg (Fig. 37.7) which could be deposited on land without danger of its drying out. A shell impervious to water is just as impervious to sperm and the development of the shelled egg coincided with the development of internal fertilization.

The extra-embryonic membranes

The embryo developing within the egg produced four extra-embryonic membranes. Protected by the fluid contained within the **amnion,** it secured food through the **yolk sac,** breathed through the **chorion** and **allantois,** and stored its metabolic wastes in the sac formed by the allantois.

Other adaptations to living on land

Probably these earliest reptiles, whose short legs extended sideways from the body, still spent most of their time in the water and simply laid their eggs on land where they could be better hidden from predators. With the increasing dryness of the Permian, however, other modifications for dry-land living evolved. Development of a dry skin enabled them to leave the water safely. But dry skin could not be used for gas exchange. Improved lungs, inflated by expansion of the rib cage, solved this problem. A partial septum in the ventricle reduced the mixing of oxygenated with deoxygenated blood and thus improved the efficiency of circulation. The success of these evolutionary developments was immediate. The stem reptiles underwent an adaptive radiation, producing five major lines of descent (Fig. 37.8).

The ancestors of the mammals

1. Although the **pelycosaurs,** too, probably spent most of their time in the water, their legs were positioned under, rather than at the sides of, the body. This position allowed for more rapid and effortless running on land.

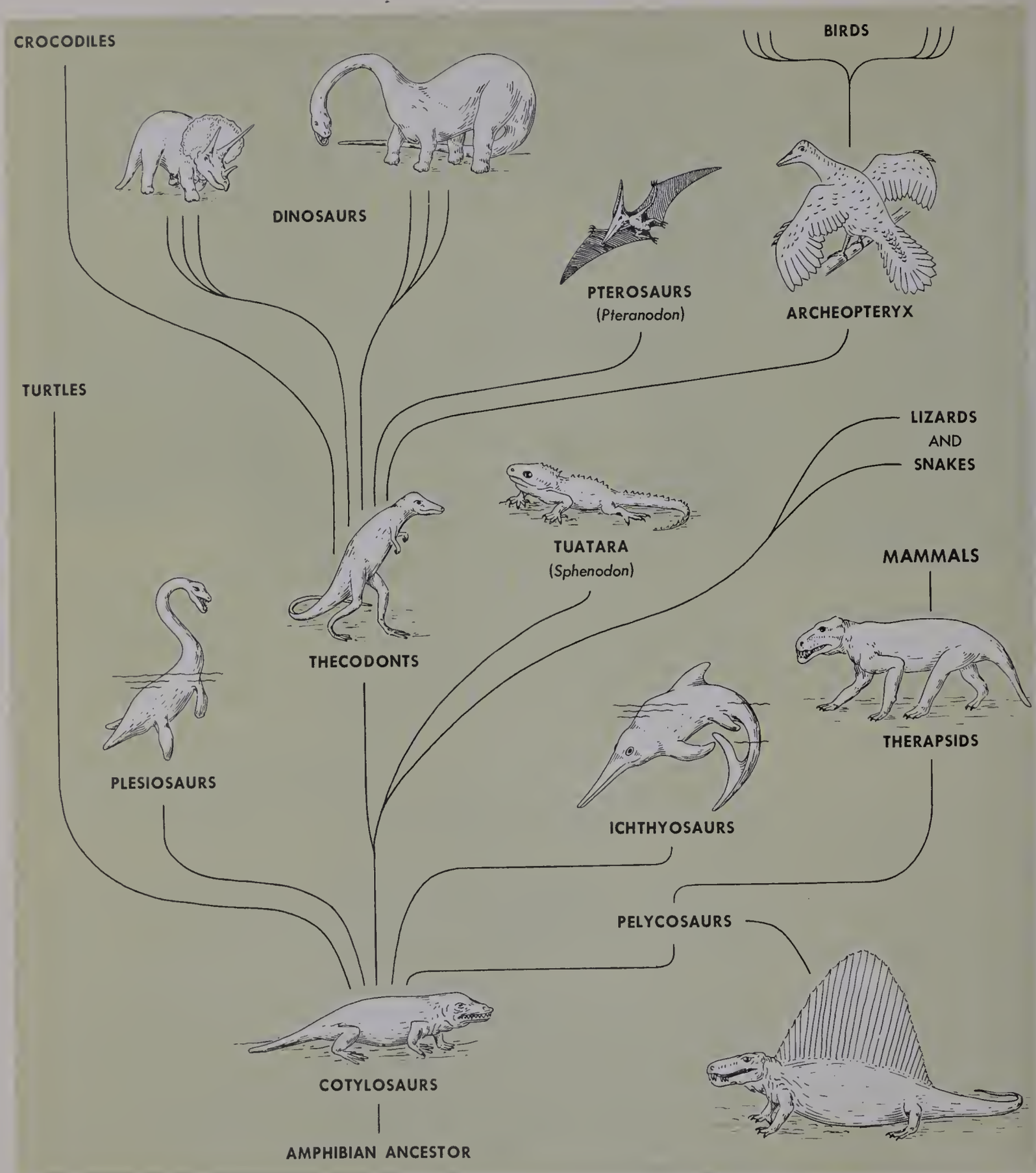


Fig. 37.8 The evolution of the reptiles.

From the pelycosaurs evolved a group of small, active, land-dwelling reptiles, the **therapsids.** At the start of the Mesozoic, these were the most abundant of the reptiles, but they soon were overshadowed in importance by other groups. But it was only a temporary (about 100 million years) eclipse, since the descendants of the therapsids, the mammals, ultimately inherited the earth.

Despite their heritage of land adaptations, many reptiles have elected to live in an aquatic environment

2. The **turtles.** Although the evolution of the reptiles produced animals capable of living on dry land, many of them did not do so. From their origin early in the Mesozoic era right down to the present time, most turtles have lived in either fresh water or the ocean. Despite their habitat, they have not abandoned their heritage of land adaptations. They breathe air (although the ribs now form the bony box covering the animal and hence cannot help to ventilate the lungs) and lay shelled eggs on land. It is interesting to realize that while our terrestrial toads are returning to fresh water each spring to lay their eggs, the freshwater turtles are crawling up on land to scoop out a hole in sand or soil in which to lay theirs. Although never a prevalent group, the turtles deserve recognition for still being with us after over 200 million years on the earth, a span of time which has seen most of their early reptile contemporaries vanish.

3 and 4. Both the **plesiosaurs** and **ichthyosaurs** (Fig. 37.8) were marine reptiles which flourished during the Jurassic but became extinct by the end of the Mesozoic era. They were undoubtedly fisheaters and quite specialized for life in the sea. In fact, their finlike appendages were so unsuited for locomotion on land that the ichthyosaurs, at least, took to retaining their eggs within the mother's body rather than laying them on land. The young were born alive and active, as many shark young are born today.

The significance of uric acid excretion

5. The remaining line of descent from the stem reptiles had one further modification for land living. This was the ability to convert nitrogenous waste into the almost-insoluble compound, uric acid. As you learned in Chapter 15, this physiological modification allows potentially toxic nitrogenous wastes to be excreted safely and with almost no loss of water. Some uric acid is filtered at the glomeruli, but most of it is excreted directly into the tubules. In fact, in the interests of water conservation, the glomeruli are reduced to the point where they produce little more nephric filtrate than is needed to flush the uric acid into the cloaca. Here even this small amount of water is largely reabsorbed. The residue of uric acid is a whitish paste that passes out with the feces. The ability of this group of reptiles and their descendants to excrete their nitrogenous wastes in this manner has almost totally freed them from dependence on drinking water. The water in their food plus that produced by cellular respiration is usually sufficient for their needs.

Evolution in this group of reptiles soon diverged into two separate lines. One group of descendants was lizardlike in appearance. The rare tuatara of New Zealand (Fig. 37.9) is a living remnant of these animals. Our modern **lizards** (Fig. 37.10), which first appeared in the Jurassic, are also





Fig. 37.9 The tuatara (*Sphenodon*). This "living fossil" is found on a few small islands near the coast of New Zealand. (Courtesy of the New York Zoological Society.)

Fig. 37.10 The Komodo monitor lizard, a native of Malaya, is the largest of the lizards. This specimen weighed over 200 pounds. (Courtesy of the New York Zoological Society.)

Fig. 37.11 The American alligator is *able* to move rapidly on land although it usually does not. (Courtesy of the American Museum of Natural History.)



Lizards and snakes

descendants although not quite in the same genealogical line. They are still important colonizers of the deserts and forests of warm parts of the world. One group of Cretaceous lizards became burrowing animals. Their legs eventually disappeared and thus the **snakes** were established. (Vestigial remnants of hind legs can still be detected in the boa and python.) Although they are able to survive in temperate regions by hibernating during the winter, the snakes also have been more successful in the subtropical and tropical regions of the world.

Thecodonts were bipedal

The **thecodonts** made up the second branch of the uric-acid-excreting, fully terrestrial reptiles. These animals were able to run quickly over the land by rising up on their hind legs and using their long tail for balance. The hind legs became larger and more powerful than the front. The great agility of these animals, combined with their tolerance of dry conditions, provided an enormously successful evolutionary plan. From them evolved five additional orders of reptiles. The members of this remarkable adaptive radiation are often referred to as the **ruling reptiles** because they completely dominated both land and air during the rest of the Mesozoic era.

a) The **crocodiles.** The crocodiles and alligators abandoned the bipedal locomotion of their thecodont ancestors but retained (and still have) the larger hind limbs. Although specimens in zoos do not often do so, these animals are capable of moving quickly with the entire torso lifted off the ground (Fig. 37.11). They are the only reptile descendants of the thecodonts that have not become extinct.

b) and c) The **dinosaurs.** Late in the Triassic, two orders of dinosaurs appeared, each of which underwent an extraordinary adaptive radiation. Throughout the remainder of the Mesozoic era, the earth was populated by dinosaurs of every size, shape, and description. Many of the larger forms were so huge that they probably were restricted to wallowing about in marshes, letting the buoyancy of the water support some of their body weight. The abundance and habitat of these forms have given us a remark-

All the dinosaurs became extinct at the end of the Mesozoic era



Fig. 37.12 Fossil skeleton of *Tyrannosaurus rex,* one of the largest bipedal dinosaurs. Note the small forelimbs of this carnivore. (Courtesy of the American Museum of Natural History.)

ably complete fossil record of them. The discovery and mounting of fossil dinosaurs was the most active branch of paleontology for many years and captured the imagination of people everywhere. When we look at the reconstructed skeletons of such forms as *Tyrannosaurus* (Fig. 37.12), which was 47 feet long and stood 19 feet high (note the greatly reduced forelimbs), and *Brachiosaurus,* which probably weighed close to 50 tons, we can well understand why. Although representing only two out of some 15 orders of reptiles in existence then, the dinosaurs alone fully justify our calling the Mesozoic era the "Age of Reptiles."

Pterosaurs were flying reptiles

d) and e) Two groups of Mesozoic reptiles took to the air. The two-legged gait of the thecodonts had freed the forelimbs for use as wings. At first these probably were used only for gliding but later sustained flight became possible. One of these groups, the **pterosaurs,** ruled the air throughout most of the Mesozoic era. *Pteranodon* (Fig. 37.8), with its wingspread of 27 feet, was the largest member of the order.



Fig. 37.13 Cast of fossil of *Archeopteryx.* The long tail, teeth, and claws on the wings are reptilian features. The feathers, clearly visible, cause it to be classified as a bird. (Courtesy of the American Museum of Natural History.)

The second group of reptiles that took to the air developed a modification not found in the pterosaurs: **feathers.** These scaly outgrowths provided a broad, light, but strong, surface for the wings. They also provided heat insulation for the body, making possible the development of a constant body temperature. These thecodont descendants were the first birds.

Archeopteryx: *a "no-longer-missing" link*

The discovery of *Archeopteryx* in Jurassic rock (Fig. 37.13) has provided us with one of the best examples of a "missing link." This creature possessed feathers, and thus we may arbitrarily call it a bird. But its relationship to the reptiles is obvious. The rather rudimentary wings had claws. There were teeth in the mouth, and it had a long tail. These are reptilian features no longer found in living birds.

Although they were well established by the end of the Mesozoic era, it was in the Cenozoic that the birds underwent such an extensive adaptive radiation. The large number of species and their wide distribution attest to their success.

The structure and physiology of birds is adapted in a variety of ways to efficient flight. Chief among these, of course, are the wings. Although wings now enable birds to travel long distances in search of suitable and abundant food, they probably first arose as an adaptation which helped them escape from predators. The presence of wingless birds in the Antarctic, New Zealand, and other regions where predators are rare gives indirect evidence of this.



Fig. 37.14 Skeleton of a pigeon. Note the large sternum to which the flight muscles are attached. Compare the tail with that of *Archeopteryx* (Fig. 37.13).

An efficient bird, like an efficient airplane, needs to be light and powerful. Lightness has been achieved by feathers, hollow bones, and a single gonad (in the females) which enlarges and is active only during the breeding season. Loss of teeth has reduced the weight of the head. Their function has been taken over by the gizzard which is located near the center of gravity.

Power is achieved by large breast muscles attached to a greatly enlarged sternum (Fig. 37.14). Birds also have a four-chambered heart, and its efficiency has permitted the development of a fixed body temperature (homeothermy). Homeothermy, in turn, permits a high metabolic rate under all environmental temperatures. Birds can remain active in cold weather, unlike their reptile cousins, which become sluggish as the temperature falls. The high metabolic rate reflects a rapid release of energy for flying. Birds secure their energy from concentrated foods, particularly those rich in fats such as seeds, insects, and other animals.

The end of the Mesozoic era was marked by great geological and biological changes on the earth. We are sure that the two were interrelated although as yet we can only speculate as to how. Geologically, this period was marked by an uplifting of mountain ranges in several parts of the world. The Rockies, the Andes, and the Himalayas achieved their present eminence at this time. Biologically, it was marked by the extinction of the reptilian orders that had flourished during the Mesozoic. By the dawn of the Cenozoic, the plesiosaurs, the ichthyosaurs, the pterosaurs, and every

In the Cenozoic, birds and mammals underwent an adaptive radiation as they moved into the niches left vacant by the reptiles

single dinosaur had vanished from the face of the earth. While we do not yet understand just why these reptiles were unable to survive the geological changes of those times, we do know that the widespread extinction of the reptiles opened up habitats on land, in the air, and in the waters of the earth. Ready to move into these habitats were the birds and the mammals.

37.8 CONTINENTAL DRIFT

One geological change that began early in the Mesozoic era and has continued to the present is the drifting of the continents over the surface of the earth. The existence of such a phenomenon, though long suspected, is only now beginning to be widely appreciated. It provides at last a satisfying explanation for a number of puzzling facts, both geological and biological.

Was there only one continent at the dawn of the Mesozoic era?

Each of these observations lends support to the idea that at the start of the Mesozoic era, all the continents were attached to one another in a single land mass, which has been given the name Pangaea (Fig. 37.15). During the



Fig. 37.15 Pangaea. Reconstruction of the single land mass thought to have existed 200 million years ago during the Triassic period and from which our present continents were formed. The reconstruction is based on a computer-generated fit of the continents as they would look if the sea level were lowered 6,000 feet. By the start of the Jurassic period, 180 million years ago, Pangaea had split into Laurasia to the north and Gondwana to the south. There is considerable evidence that continental drift has continued to the present time and, indeed, is still continuing. (Adapted from the data of R. S. Dietz and J. C. Holden.)

Triassic, Pangaea began to break up, first into two major land masses: Laurasia in the northern hemisphere and Gondwana in the southern hemisphere. Although the exact timetable is still uncertain, the present continents separated at intervals throughout the rest of the Mesozoic and through the Cenozoic era, ultimately reaching the positions that they occupy today. Although a careful study of the physical evidence for continental drift is beyond the scope of this book, let us at least glance at some of it.

Geological evidence for the existence of Pangaea

The shapes of the present continents have long suggested to astute observers that they might at one time have been in direct contact. The east coast of South America and the west coast of Africa are strikingly complementary. This complementarity is even more dramatic when one attempts to fit the continents together using the edges of the continental shelf (at 500 or 1000 fathoms) as the boundaries rather than the present shorelines (Fig. 37.15).

If the continents were once a single land mass, we would expect to find similar geological features in areas now separated but once presumed to have been in contact. This is indeed the case. In both mineral content and age (as determined by isotope dating), the rocks in a small area on the east coast of Brazil match precisely those found in Ghana on the west coast of Africa. The low mountain ranges and rock types in New England and eastern Canada appear to be continued in parts of Great Britain, France, and Scandinavia. India and the southern part of Africa both show evidence of periodic glaciation during Paleozoic times. This is a remarkable finding, indeed, for land that now lies close to the equator. Not only do the *patterns* of glacial deposits match in each of these areas, but they also match those found in South America, Australia, and Antarctica.

When rocks containing iron ores are first formed, they become permanently magnetized in the direction of the earth's magnetic field at that time. The study of such rocks formed at different times in the earth's history in different areas of the earth has revealed them to be magnetized in directions that corroborate the story of continental wandering we have been discussing. Faced with such an impressive body of evidence for the existence of continental drift, is there any mechanism which would provide the forces necessary to move the continents about? One mechanism that has been postulated involves spreading of the floors of the oceans; it has been given the name **plate tectonics.** A large body of geophysical evidence has been gathered in recent years—ranging from the analysis of earthquakes to measurements of the magnetism and topology of the ocean floors—that supports the concept of plate tectonics and thus provides a plausible mechanism for continental drift. Several articles and a book in which you may examine this evidence are cited at the end of the chapter.

Plate tectonics: a plausible mechanism for continental drift

What does continental drift tell us about the history of animal and plant evolution and vice versa? The hypothesis that a single land mass has broken up into the present continents explains many paleontological puzzles. It explains, for example, why certain fossil reptiles that are found in South Africa are also found in Brazil and Argentina. It makes sense of the

Biological puzzles solved by the Pangaea hypothesis

discoveries in 1969 and 1970 of fossil amphibians, cotylosaurs, and therapsids in Antarctica. In many cases the species that were identified there are also present as fossils in South Africa, India, and China. It even explains why the humble earthworm of eastern North America has such close relatives in western Europe but not in western North America. These are only a few of many examples. The distribution of plants and animals from Mesozoic times to the present, on the one hand, can best be understood in terms of a gradual fragmentation of Pangaea into the continents of today; on the other, it reinforces the evidence acquired by geophysicists that our continents have indeed been on the move.

37.9 THE EVOLUTION OF THE MAMMALS

We think that the first mammals arose late in the Triassic period from therapsid (Fig. 37.8) ancestors. They were small but very active animals whose diet probably consisted chiefly of insects. Associated with their active life was their ability to maintain a fixed body temperature (homeothermy). As in the birds (which did not appear until the Jurassic) this went hand in hand with the development of a four-chambered heart and completely separate oxygenating and systemic circuits. Conservation of body heat was made possible by the development of **hair.** While the earliest mammals laid eggs as did their reptile ancestors, the young, after hatching, were nourished with **milk** secreted by glands in the mother's skin.

The diagnostic traits of mammals: hair and milk

In contrast to their reptile ancestors, the teeth of the mammals became specialized for cutting (incisors), tearing (canines), and grinding (molars) their food. The gray matter of the cerebrum, which is covered by white matter in the reptiles, grew out and over the surface of the brain. This modification was to have far-reaching consequences.

Evolution of the earliest mammals took several different routes. Of the groups that evolved, only three have survived to the present. Each of these was distinguished by the way in which the young were cared for during embryonic development. One group, the **monotremes,** continued to lay eggs as the therapsids had. The duckbill platypus and the spiny anteaters (echidnas, Fig. 21.8) are the only descendants of this group still on the earth. In the second group, the **marsupials,** the young are retained for a short period within the reproductory tract of the mother. During this brief time, some nourishment is secured by means of the yolk sac which grows into the wall of the uterus. At a very early stage of development, though, the young are born. They then crawl into a pouch on the mother's abdomen and attach themselves to a milk-dispensing nipple. Development is completed here.

The monotremes still lay shelled eggs

Marsupials

The earliest marsupials probably resembled our opossum. They *may* have evolved in North America but, while Gondwana still existed, spread into the areas that were to become South America, Australia, and New Zealand. Their presence in South America and Australia is difficult to explain without assuming a unified land mass during at least the early part

of the Mesozoic. We may with some confidence predict that fossil marsupials will some day be found in Antarctica.

In the relative isolation of Australia and South America, the marsupials underwent elaborate adaptive radiations. But the reuniting of North and South America through the isthmus of Panama some two million years ago brought the third and most advanced group of mammals, the **placental mammals,** into direct competition with the South American marsupials, all but 69 species of which have since become extinct. Only in Australia and New Zealand, areas where placental mammals have only just recently gained entrance, does a rich diversity of marsupials survive today. The monotremes also survive only in these areas.

Placental mammals

The placental mammals retain their young within the uterus of the mother until development is well advanced. There is little yolk in the egg but the extra-embryonic membranes form an umbilical cord and placenta through which the developing young are able to secure nourishment directly from the mother.

1. Insectivora: shrews, moles
2. Scaly Anteaters
3. Edentata: armadillos
4. Chiroptera: bats
5. Rodentia: squirrels, rats, mice, beaver
6. Lagomorpha: rabbits and hares
7. Carnivora: lion, cats, dogs, skunk, walrus, sea lion
8. Odd-toed Ungulates: zebra, horse, rhinoceros.
9. Even-toed Ungulates: camels, elk, giraffe, bison, sheep, hippopotamus
10. Cetacea: whales, dolphins, porpoises
11. Sirenia: manatee, dugong
12. Proboscidea: elephants
13. Aardvarks
14. Primates: lemurs, New World monkeys, Old World monkeys, great apes, man

Fig. 37.16 Orders of placental mammals and representatives of each.

For some 70 million years in the Mesozoic era, the placental mammals were represented by just a single order. However, by the end of the second epoch, the Eocene, of the Cenozoic era, the placentals had radiated into at least fourteen different orders (Fig. 37.16). What explains their long period of insignificance followed by such an explosive burst into prominence? The first requirement for speciation is relaxed selection pressure, and it was not until the extinction of the ruling reptiles that the mammals secured this. With most of the reptiles gone, a variety of habitats was available for occupancy by the mammals. Ungulates replaced the herbivorous dinosaurs. Carnivores preyed upon them. The cetaceans (whales, etc.) and some carnivores replaced the plesiosaurs in the oceans. The ancestors of the bats



Fig. 37.17 A tarsier. His large, forward-looking eyes and his grasping hands are typical of the primates. (Courtesy New York Zoological Society.)

even found a new habitat. With the aid of echo location, they were able to prey upon night-flying insects. This ability and the lack of competition in this area have resulted in their order's becoming second only to the rodents in number of species.

37.10 THE EVOLUTION OF THE PRIMATES

Primate traits

One order of placental mammals, the primates, is of particular interest to us because we are members of it. It had its origin some 60 million years ago at the very dawn of the Cenozoic. The first members were probably descended from the insect-eating placentals of the Mesozoic. During the Paleocene epoch, these evolved into a group of tree-living animals of which the lemurs and tarsier of today are the little-changed descendants. Grasping hands (with nails) and good vision are both of great value for life in the trees, and, as Fig. 37.17 makes clear, these animals are equipped with both. Not only is travel through the tree trops made easier and safer, but these features pay an extra dividend in permitting the animal to feed while keeping a constant watch for enemies.

By the Oligocene, these early primates had split into three separate stocks, the "New World" monkeys, the "Old World" monkeys and the ancestors of the apes. Their most significant evolutionary advance was the development of stereoscopic ("3D") vision as well as good color vision.

The New World monkeys seem always to have been confined to tropical America. The other two groups spread over Africa, parts of Europe, and Asia.

Hominids and pongids

The ancestors of the apes differed from the Old World monkeys in developing longer arms, a more erect posture and in losing their tails. These modifications reflected their method of locomotion: swinging from branch to branch in the trees. In the Pliocene, they subdivided again into the "hominids," who came down out of the trees and took to walking over the plains in search of food, and the true apes (pongids), who remained in the trees. From these latter forms have come the so-called "great apes," the gorilla, chimpanzee, and orangutan (Fig. 37.18).

37.11 THE EVOLUTION OF MAN

Although we are certainly descendants of the early hominids, the scarcity of fossil remains makes it difficult to trace our genealogy exactly. Perhaps as early as the Pliocene (four to five million years ago), and surely throughout the Pleistocene, two independent "man-ape" (australopithecine) populations seem to have coexisted: a stocky form now placed in the genus *Paranthropus* and a smaller, slightly built form now often called *Homo africanus.* While *Paranthropus* may have used crude tools, *Homo africanus* was probably more inventive in this respect.

Paranthropus *and* Homo africanus *coexisted*

Now only one adaptation remained to complete the fabrication of man: the great enlargement of the cerebral cortex which enabled him to

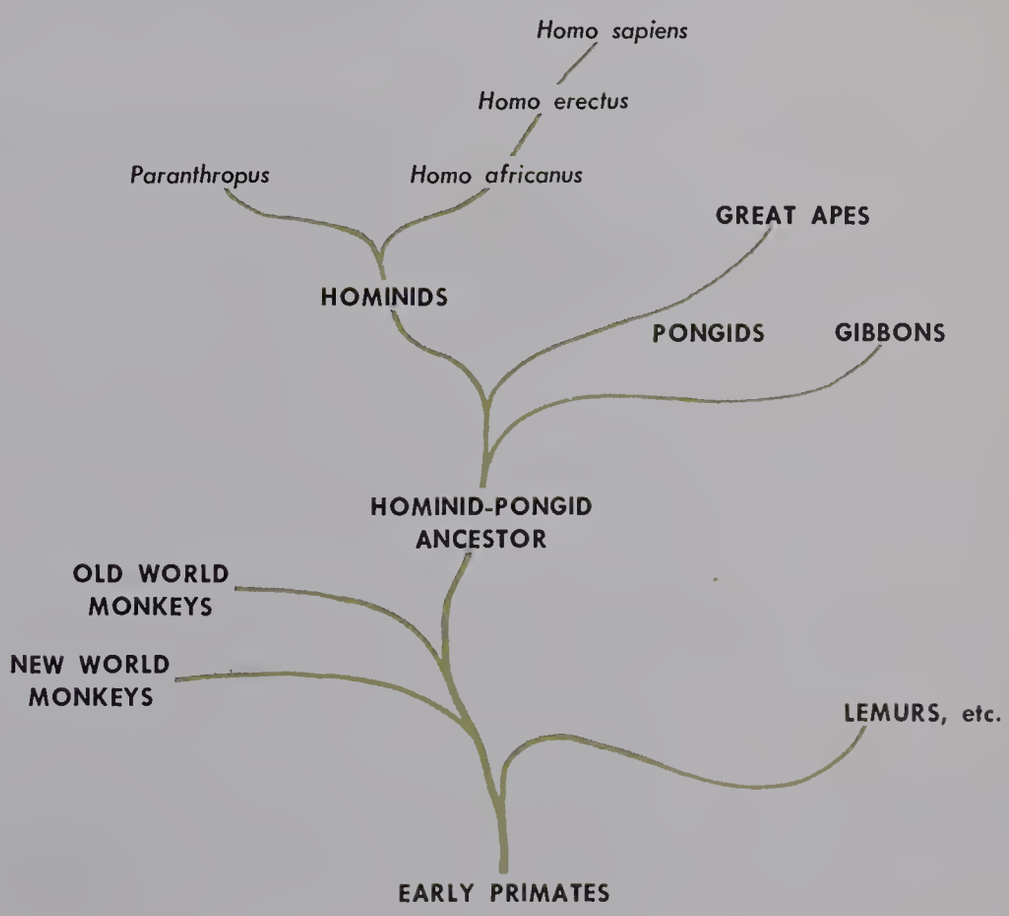


Fig. 37.18 The evolution of the primates. Both *Paranthropus* and *Homo africanus* are assigned by some workers to the genus *Australopithecus.*

Homo erectus

exploit fully his tool-making capabilities. By five million years ago, *Homo erectus* existed throughout much of Africa and Asia (e.g. Java man and Peking man). His cranial capacity averaged some 950 cm^3, as compared to the 450 cm^3 average of *Homo africanus* (and overlapping the 800–2000 cm^3 range found in modern man). On the basis of archeological data and gene frequencies in present human populations (e.g. for blood groups) we assume that *Homo erectus* became isolated in three geographically separated subpopulations: Negroid (in Africa), Caucasoid (in the Near East and, later, Europe), and Mongoloid (in Asia).

Starting on the path to subspeciation

Thus may have begun the process of subspeciation that gave rise to the racial groups of today. We have no reason to think that the process operated any differently from the process that has given rise to the seven subspecies of *Geothlypis trichas* in North America (Fig. 32.4). The skills of *Homo erectus* must have made him a potent competitor against *Paranthropus,* and *Paranthropus* appears to have eventually become extinct.

If this theory of subspeciation in *Homo erectus* is correct, then we must assume that modern man, *Homo sapiens,* represents the outcome of evolutionary trends that occurred in all the *Homo erectus* groups wherever they were. Such an occurrence would help explain the continuum that archeologists find between the features of *Homo erectus* and those of *Homo sapiens* —in Europe, in Africa, and in Asia.

Man in the New World

There is no evidence that any hominids existed in the Western Hemisphere until *Homo sapiens* arrived via the land "bridge" which has periodically connected Asia and North America in the Bering Strait region. By 22,000 years ago (perhaps even earlier) humans were well established in North, Central, and South America. These were the Indians whom Columbus found when, in 1492, the colonization of the New World began from Europe and Africa.

Gene migration in human populations

We have seen that formation of geographically isolated subspecies is a first step toward speciation. Did *Homo erectus* and his successor *Homo sapiens* continue along the path? Clearly, the answer is no. Humans have always been extraordinarily mobile. Various human populations have migrated repeatedly throughout virtually the entire world. Wherever one such population has met another, some interbreeding has occurred and thus some fusing of their respective gene pools. While cultural barriers to interbreeding have often existed, there is absolutely no evidence that the early steps of subspeciation ever proceeded to the point where any genetic barriers to interbreeding had become established. *Homo sapiens* is today a single species, albeit of considerable genetic diversity, of worldwide distribution. The partial fusing of gene pools of populations that had for a time been genetically isolated has occurred so many times and in so many places that it is an incredibly complex task to try today to unravel the history of migrations and consequent gene flow that has been so characteristic of human evolution.

Evolution: teleology or opportunism?

In tracing the evolutionary history of the animals, it is tempting to see in the story a guiding hand working out the destiny that ultimately produced man from the first flatworms. According to such a view, lungs,

limbs, and the shelled egg evolved so that animals could colonize the land, and grasping hands arose so man could become a tool user. Such a view of evolution is called teleological. It interprets the process of evolution as one of working toward a predetermined goal. There is no evidence in the fossil record to support such a view. On the contrary, we see that the evolution of lungs and limbs was the result of selection pressure acting to enable the crossopterygians to remain in the water. The shelled egg probably evolved because the aquatic, early reptiles that produced it could hide it on land out of sight of hungry aquatic predators. The grasping hand evolved in response to the needs of life in the trees. But, note that each of these adaptations, which arose in response to certain selection pressures, then provided a plan that enabled a new environment to be colonized. The amphibians found the earth filled with plants and insects. So did the more efficient reptiles, and the amphibians began a decline which has continued to this day. Two-thirds of the history of the mammals was spent in obscurity. With the widespread extinction of reptiles, however, the land was left open for the mammals to begin their period of dominance which still continues. Could the reptiles ever take over again? Certainly not until or unless the now-dominant mammals disappear in large numbers from the face of the earth. The fossil record is filled with examples, both major and minor, of animal groups which arise, flourish for a time, finally become extinct and, in so doing, pave the way for an adaptive radiation of some other animal group. It is a story of adaptations, evolving to meet some immediate need, which by chance alone turn out to open new opportunities, new, less crowded ways of life to their owners.

EXERCISES AND PROBLEMS

1. In what ways does the evolutionary history of the amphibians parallel that of the mosses? In what ways does it differ from it?
2. Summarize the specific structural and physiological modifications that make birds so successful in their way of life.
3. Summarize the adaptations which have made reptiles more successful at colonizing the land than the amphibians have been.
4. The Java man was once classified as *Pithecanthropus erectus*. Now most anthropologists classify him as *Homo erectus*. What change in their thinking does this reflect?
5. On the basis of the evidence presented in this chapter, would you say that adaptive radiations have had their origin in highly successful, specialized groups of animals? Defend your position.
6. What features of man are unique to him?
7. Would you consider man as the most specialized of the mammals? Defend your view.
8. Should caecilians be considered primitive amphibians? Defend your view.

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GLOSSARY

Guide to Pronunciation*

ə —b**a**nana, c**o**llect, **a**but
ər —op**er**ation, f**ur**ther
ä —b**o**ther, c**o**t
ŋ —'sing\siŋ\ singer\ 'siŋ-ər\
ȯ —s**aw**, **a**ll, gn**aw**
yü—**y**outh, **u**nion, c**u**, f**ew**, m**u**te
' —primary accent (before main accented syllable)
, —secondary accent
\ —slant line, in pairs, marks beginning and end of a transcription

absorption spectrum Electromagnetic spectrum whose intensity at each wavelength is a measure of the amount of energy at the wavelength that has passed through a selectively absorbing substance.

acetylcholine \ə-ˌsēt-ᵊl-'kō-ˌlēn\ Organic compound secreted at the ends of many neurons. A neurohumor.

acid [L. *acidus,* sour] Molecule or ion that liberates protons, usually in water.

actinomycin D \ˌak-ti-ˌnō-'mīs-ᵊn\ An antibiotic isolated from soil bacteria that blocks DNA-dependent RNA synthesis.

action spectrum Rate of physiological activity plotted against wavelength of light.

active transport Transfer of a substance across a cell membrane from a region of low to one of high concentration. Requires energy.

adaptation [L. *adaptare,* to fit] Any characteristic of an organism that contributes to its survival in its environment.

adaptive radiation Evolution, from a single ancestral species, of a variety of descendant species adapted to different ways of life

adenosine triphosphate (ATP) \ə-'den-ə-ˌsēn-ˌtrī-'fäs-ˌfāt\ Organic compound that is the immediate source of energy for the activities of the cell.

adhesion Force of attraction between unlike molecules.

adventitious root Root that arises from a stem or leaf.

aerobic \ˌa-(ə-)'ro-bik\ [Gk. *aeros,* air; *bios,* life] Requiring the presence of free oxygen (O_2).

alga \'al-gə\ A photosynthetic, often plantlike, organism generally found growing in aquatic or damp locations.

allantois \ə-'lant-ə-was\ [Gk. *allas,* sausage] Extraembryonic membrane of reptiles, birds, and mammals that forms a pouch growing out of the posterior part of the alimentary canal.

allele \ə-'lē(ə)l\ Alternative form of a gene that may occur at a given gene locus.

allosteric \ˌal-ə-'stir-ik\ Refers to a change in the properties (probably including shape) of a protein following the binding of a small molecule to a site on the protein other than its active site.

amino terminal The end of a polypeptide chain that has a free amino group ($-NH_2$) attached to the backbone of the chain. Synthesis of polypeptide chains proceeds from the amino terminal to the carboxyl terminal.

amniocentesis \'am-nē-ō-ˌsen-'tē-ses\ Removal of amniotic fluid from a pregnant woman in order to study its composition and/or culture the cells contained in it.

amnion \'am-nē-ˌän\ Extraembryonic membrane of reptiles, birds, and mammals that encloses the embryo in a fluid-filled sac.

amoeba \ə-'mē-bə\ A unicellular protozoan that moves by means of pseudopodia.

amphetamine \am-'fet-ə-ˌmēn\ A drug that is similar in molecular structure to adrenaline and noradrenaline and shares their stimulatory properties.

amylase \'am-ə-ˌlās\ [L. *amylum,* starch] An enzyme that digests (hydrolyzes) starch.

anabolism \ə-'nab-ə-ˌliz-əm\ Constructive metabolism in which complex substances are synthesized from simpler ones.

anaerobic \ˌan-ə-'rō-bik\ [Gk. *an,* not + aerobic] Not requiring the presence of free oxygen.

analogous \ə-'nal-a-gəs\ [Gk. *analogos,* proportionate] (Of organs in different species), having a similar function but a different structure and embryonic development.

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androgen \'an-drə-jən\ One of a group of sex hormones of male vertebrates which promotes the development of the secondary sex characteristics.

anemia [Gk. *anaimia,* bloodlessness] Deficiency of red blood cells or hemoglobin in the blood.

anesthetic \ˌan-əs-'thet-ik\ A substance that produces loss of sensation.

antibody A protein, produced by a vertebrate, that can unite with a specific antigen.

antigen \'ant-i-jən\ Macromolecule (usually a protein or polysaccharide) which, when introduced into the body of an animal it is foreign to, stimulates the formation of antibodies.

antigenic determinant Any portion of an antigen that combines with the active site of an antibody.

antitoxin Mixture of antibodies formed in response to a toxin.

aquifer \'ak-wə-fər\ A layer in the earth that is saturated with water.

archenteron \är-'kent-ə-ˌrän\ Central cavity of the gastrula of an embryo, ultimately becoming the alimentary canal.

arthropod A member of the phylum of invertebrate animals characterized by jointed legs and exoskeleton. Includes insects, arachnids, and crustaceans.

ascus \'as-kəs\ [Gk. *askos,* wineskin, bladder] Tubular spore sac of ascomycetes in which (usually) 8 ascospores are produced.

asexual \(')ā-'seksh-(ə-)wel\ **reproduction** Reproduction without the union of gametes (or any nuclear material).

atom \'at-əm\ [Gk. *atomos,* indivisible] Smallest particle of an element that can enter into combination with other elements.

atomic weight unit One-twelfth the weight of an atom of carbon-12. Also called the dalton.

auto-immune disease Disease characterized by an individual's mounting an immune response against constituents of his own tissues.

autosome \'ot-ə-ˌsōm\ Any chromosome that is not a sex chromosome.

autotrophic \ˌȯt-ə-'träf-ik\ Capable of synthesizing organic compounds from inorganic raw materials.

auxin \'ȯk-sən\ [Gk. *auxein,* to increase] Plant hormone that, among other effects, promotes cell elongation.

axon \'ak-ˌsän\ Single extension of a neuron (usually long and often branched), which conducts nerve impulses away from the dendrites.

bacteriophage \bak-'tir-ē-ə-ˌfāj\ [bacterium + Gk. *phagein,* to eat] Virus that infects bacteria.

barbiturate \bär-'bich-ə-rət\ Any of several hundred derivatives of barbituric acid used as sedatives (e.g. phenobarbital).

base Molecule or ion that can take a proton from an acid.

basidium \bə-'sid-ē-əm\ Club-shaped spore-producing structure of basidiomycetes on which four basidiospores are formed.

B cell An immunologically active cell (lymphocyte) that is not dependent on the thymus gland for its activity. Antibody-*secreting* cells (plasma cells) are derived from B cells.

beta-galactosidase \'bāt-ə ˌgə-ˌlak-tə-'sī-ˌdās\ An enzyme ("lactase") that hydrolyzes the disaccharide lactose.

bioassay \ˌbī-(ˌ)ō-'as-ˌā\ Quantitative determination of the strength of a biologically active substance from its effect on a living organism.

biomass The total amount of living matter in a given population of organisms.

biome \'bī-ōm\ A distinctive plant and animal community produced and maintained by the climate. The coniferous forest region of North America constitutes a single biome (the taiga).

biosphere The part of our planet in which life exists and with which it exchanges materials. Includes a small part of the lithosphere (the solid earth) and large parts of the hydrosphere (water) and atmosphere.

blastula \'blas-chə-lə\ Early stage of animal development in which a single (usually) layer of cells surrounds a fluid-filled cavity (the blastocoel), thus forming a hollow ball.

botany \'bät-ᵊn-ē\ [Gk. *botanē,* pasture, herb] The study of plants.

branchial grooves \'braŋ-kē-əl\ Series of external, paired grooves in the neck region of verte-

brate embryos that correspond in position to the outpocketings of the pharynx (the gill pouches).

budding Asexual reproduction in which a new organism develops from an outgrowth of the parent.

calorie [L. *calor,* heat] The amount of heat required to raise the temperature of 1 gm of water 1°C. When capitalized, a unit of heat 1000 times larger than the above.

cambium \'kam-bē-əm\ Layer of meristematic cells in the roots and stems of many tracheophytes that produces secondary xylem and phloem.

cancer \'kan-sər\ Any of a group of diseases characterized by an uncontrolled proliferation of cells.

carbohydrate \ˌkär-bō-'hī-ˌdrāt\ Organic compound of carbon, hydrogen, and oxygen, generally with a 2:1 ratio of hydrogen atoms to oxygen atoms. Sugars, starches, and cellulose are carbohydrates.

carboxyl terminal The end of a polypeptide chain with a free carboxyl (–COOH) group attached to the backbone of the chain. Synthesis of a polypeptide chain proceeds from the amino terminal to the carboxyl terminal.

carcinogen \kär-'sin-ə-jən\ A cancer-inducing substance.

catabolism \kə-'tab-ə-ˌliz-əm\ Destructive metabolism in which complex molecules are broken down into simpler ones, with the liberation of energy.

catalyst \'kat-ᵊl-əst\ Substance that accelerates the rate of chemical reaction without being used up in the process.

cell-mediated immunity An immune response (e.g. graft rejection) that is dependent on the presence of specifically-sensitized (i.e. able to recognize a particular antigen) lymphocytes.

cephalization \ˌsef-ə-lə-'zā-shən\ Evolutionary tendency toward the concentration of sense receptors and central nervous system at the anterior end of an animal.

cephalothorax \ˌsef-ə-lō-'thō(ə)r-ˌaks\ Fused head and thorax found in arachnids and many crustaceans.

chalone \'kal-ˌōn\ A substance secreted by a tissue that inhibits mitosis in that tissue.

chaparral \ˌshap-ə-'ral\ The scrub forest biome found in southern California.

chemoautotrophic \ˌkem-ō-ˌȯt-ə-'träf-ik\ Autotrophic, using energy secured by oxidizing some inorganic substance. Characteristic of certain bacteria.

chitin \'kīt-ᵊn\ Nitrogen-containing polysaccharide that forms the exoskeleton of arthropods and the cell walls of many fungi.

chlorophyll [Gk. *chloros,* green] Green pigment used in photosynthesis.

chloroplast \'klōr-ə-ˌplast\ Plastid containing chlorophyll.

cholesterol \kə-'les-tə-ˌrȯl\ The most abundant steroid in the human body. It probably serves as the starting material for the synthesis of the other steroids found in the body.

cholinesterase \ˌkō-lə-'nes-tə-ˌrās\ Enzyme that hydrolyzes and thus inactivates acetylcholine.

chorion \'kor-ē-ˌän\ Extraembryonic membrane of reptiles, birds, and mammals that covers the embryo and, in mammals, contributes to the formation of the placenta.

chromatography \ˌkrō-mə-'täg-rə-fē\ The process of separating the components of a mixture by their differential adsorption to an insoluble matrix (e.g. paper) as the mixture is passed through the matrix.

chromosome \'krō-mə-ˌsōm\ Elongated structures in the cell nucleus, containing DNA and protein and bearing the genes. The number in the nucleus is usually constant for each species.

circadian \ˌsər-'kā-ˌdē-ən\ [L. *circum,* round about + *dies,* day] Occurring approximately once a day.

cleavage Repeated mitotic division of the zygote which forms the many-celled blastula.

cline \'klīn\ Continuous gradation of structural or physiological differences exhibited by the members of a species along a line extending from one part of their range to another.

cloaca \klō-'ā-kə\ Posterior part of the alimentary canal into which the urinary and reproductive ducts empty in birds, reptiles, amphibians, and many fishes.

clone The descendants produced asexually from a single cell or a single organism. Characterized by an identical genetic constitution.

cnidaria \nī-'da-rēə\ [Gk. *knidē,* nettle] The phylum of animals that includes the hydra, jellyfishes, sea anemones, and corals. Formerly called Coelenterata.

codon The three adjacent bases in a molecule of DNA or messenger RNA that code for a particular amino acid.

coelom \'sē-ləm\ [Gk. *koilōma,* cavity] Main body cavity of many animals. It is lined with an epithelium derived from mesoderm.

coenocyte \'sē-nə-ˌsīt\ Mass of cytoplasm, containing many nuclei formed by the repeated division of the nucleus of a single cell with no division of its cytoplasm.

coenyzme \(')kō-'en-ˌzīm\ Organic compound that by combining temporarily with an enzyme makes it active.

cohesion Force of attraction between like molecules.

coleoptile \ˌkō-lē-'äp-tᵊl\ [Gk. *koleos,* sheath] Protective sheath surrounding the plumule of grass seedlings.

collenchyma \kə-'leŋ-kə-mə\ Supporting plant tissue consisting of living cells whose walls are thickened at the corners. Often found in young, growing stems and petioles.

colloid \'käl-ˌȯid\ [Gk. *kolla,* glue] Substance whose particles (macromolecules or aggregates of smaller molecules) range from 1 nm to 100 nm in size.

commensalism \kə-'men(t)-sə-ˌliz-əm\ [L. *com,* together + *mensa,* table] A close living relationship between two species, in which one benefits from the other without harming or benefiting it.

compound Substance that can be decomposed into simpler substances. The elements of a compound are present in definite proportions by weight.

conifer A cone-bearing gymnosperm. Includes pines, spruces, firs, etc.

conjugation Form of sexual reproduction in which genetic material is exchanged during the temporary union of two cells. Occurs in many ciliates (e.g. *Paramecium*) and some bacteria.

convergence [L. *convergere,* to turn together] Evolution of superficially similar traits in unrelated organisms that live in a similar environment.

copulation \ˌkäp-yə-'lā-shən\ Physical union of two animals during which sperm cells are transferred from one to the other.

corepressor A small molecule that joins with a repressor molecule to block gene action.

cortex \'kȯr-ˌteks\ [L. *cortex,* bark] The outer part of an organ.

covalent bond \(')kō-'va-lənt\ Chemical bond formed by one or more shared pairs of electrons.

cutin \'kyüt-ᵊn\ Waxy material secreted by the exposed epidermal cells of plants.

cytochrome \'sīt-ə-ˌkrōm\ One of several iron-containing proteins, found in mitochondria, and chloroplasts, that transfer electrons in the process of cellular respiration.

cytokinin \'sī-tō-ˌkī-nin\ One of a group of adenine-containing compounds that stimulate mitosis in plants.

cytoplasm \'sīt-ə-ˌplaz-əm\ General term for all the contents of a cell outside of the nucleus and within the cell membrane.

dalton Unit of weight equal to 1/12th the weight of an atom of ^{12}C.

deamination \(ˌ)dē-ˌam-ə-'nā-shən\ Removal of an amino ($—NH_2$) group from a compound.

dedifferentiation \(')dē-ˌdif-ə-ˌren-chē-'ā-shən\ Reversion of a specialized cell to a more generalized, embryonic type.

denaturation \(ˌ)dē-ˌnā-chə-'rā-shən\ Alteration of the physical properties and three-dimensional structure of a protein by agents too mild to break the peptide bonds.

dendrite \'den-ˌdrīt\ Branching, usually short, extension of a neuron in which the nerve impulse is generated.

denitrification Reduction of nitrates to free nitrogen (N_2).

deoxyribonucleic acid (DNA) \dē-ˌäk-sē-'rī-bō-n(y)ü-ˌklē-ik\ Nucleic acid found in chromosomes that stores the hereditary information of the organism.

dialysis \dī-'al-ə-səs\ The separation of solute molecules by virtue of their differing rates of diffusion through a semipermeable membrane.

diastole \dī-'as-tə-lē\ Phase of relaxation of the heart.

differentiation \ˌdif-ə-ˌren-chē-'ā-shən\ Structural and functional modification of an unspecialized cell into a specialized one.

diffusion Migration of molecules or ions, as a result of their own random movements, from a region of higher to a region of lower concentration.

digestion \dī-'jes(h)-chən\ Breakdown of macromolecules of food by hydrolysis.

dihybrid \(')dī-'hī-brəd\ Being heterozygous at two different gene loci.

dioecious \(')dī-'ē-shəs\ Having male sex organs on one plant, female on another of the same species. The holly is dioecious.

diploid \'dip-ˌlȯid\ Having two of each kind of chromosome (except for the sex chromosomes); 2*n*.

disaccharide \(')dī-'sak-ə-ˌrīd\ A sugar (e.g. sucrose) that can be hydrolyzed into two monosaccharides.

dissociation \(ˌ)dis-ˌō-s(h)ē-'ā-shən\ Separation of ions from a molecule or crystal lattice.

distal \'dist-ᵊl\ Situated away from the place of origin or attachment.

DNA polymerase An enzyme that catalyzes the linking together of deoxyribonucleotides to form DNA complementary to a template (either DNA or, in the case of reverse transcriptase, RNA).

ecology \ˌi-'käl-ə-jē\ Study of the interrelationships of organisms and their environment.

ecosystem \'ekō-(or ēkō-),sis-təm\ A community of organisms interacting with each other and with their nonliving surroundings.

ectoderm \'ek-tə-ˌdərm\ [Gk. *ektos,* outside + *derma,* skin] Outermost layer of cells of an animal embryo.

edema \i-'dē-mə\ [Gk. *oidema,* swelling] Abnormal accumulation of lymph in the tissue spaces.

effector \i-'fek-tər\ Body structure by which an organism acts. In man the chief effectors are the muscles and glands.

egestion \i-'jes(h)-chən\ [L. *egerere* to carry outside, discharge] Elimination of undigested materials from the alimentary canal.

electron \i-'lek-trän\ Negatively charged particle present outside the nucleus of an atom.

electronegative Having an affinity for electrons.

electropositive Having a tendency to release electrons.

element Any of about 100 substances that consist of only one kind of atom and cannot be decomposed into simpler substances.

embryo An animal or plant in an early stage of development from a zygote.

emphysema \ˌem(p)-fə-'sē-mə\ [Gk. *emphysēma,* bodily inflation] A condition of the lungs characterized by a reduction in the surface available for gas exchange.

emulsion \i-'məl-shən\ [L. *emulgere,* to milk out] Mixture consisting of droplets of one liquid suspended in a second.

endocytosis The engulfment by a cell of extracellular material accomplished by the invagination and pinching off of a portion of the cell membrane. The engulfed material is thus enclosed within a vacuole.

endoderm \'en-də-ˌdərm\ [Gk. *endon,* within + *derma,* skin] Innermost layer of cells of an animal embryo.

endosperm \'en-də-ˌsperm\ [Gk. *sperma,* seed] Nutritive tissue that surrounds and nourishes the developing embryo of seed plants.

endosymbiont An organism living within the body of its symbiotic partner.

energy [Gk. *energos,* active] Capacity for doing work.

enzyme \'en-zīm\ [Gk. *enzymos,* leavened] Protein catalyst produced by a living organism.

epicotyl \'ep-ə-ˌkät-ᵊl\ That portion of the shoot of a plant embryo or seedling above the node at which the cotyledons are attached.

epiphyte \'ep-ə-ˌfīt\ [Gk. *epi,* on + *phyton,* plant] Plant that grows entirely on another plant but for position and support only.

equilibrium State of balance between opposing actions.

estrogen \'es-trə-jən\ One of a group of female sex hormones which, among other effects, promotes the development of the secondary sex characteristics.

etiolation \'ēt-ē-ə-ˌlā-shən\ A phenomenon exhibited by plants grown in darkness, character-

ized by pale color, long internodes, and small leaves.

eukaryote \yü-'ka-rē-ˌōt\ An organism whose cells contain a membrane-bounded nucleus. Often spelled eucaryote.

eutrophication \ˌyü-ˌträf-ə-'kā-shən\ The process in which a body of water becomes enriched in dissolved nutrients.

excretion [L. *excretus,* sifted out] Elimination of metabolic wastes by an organism.

exocytosis The discharge of vacuole-enclosed materials from a cell by the fusion of the vacuole membrane with the cell membrane.

extensor \ek-'sten-sər\ A muscle that extends a limb.

extracellular fluid (ECF) The fluid which bathes cells.

fauna \'fon-ə\ Animal life in a certain environment.

feedback inhibition The inhibition of the first enzyme in a metabolic pathway by the final product of that pathway.

fermentation Anaerobic decomposition of an organic compound (e.g. glucose) by a living organism.

fetus \'fēt-əs\ [L. offspring] Unborn mammal after it has largely completed its embryonic morphogenesis and differentiation (in humans, after three months of development).

fission \'fish-ən\ [L. *fissus,* split] Asexual reproduction by division of the body into two or more equal parts.

flatworm Any member of the phylum Platyhelminthes. Includes turbellarians, tapeworms, and flukes.

flavin \'flā-vən\ [L. *flavus,* yellow] Yellow pigment that, when combined with a protein, transports electrons to the cytochromes.

flexor \'flek-sər\ A muscle that bends a limb.

flora Plant life in a certain environment.

fluorescence \(ˌ)flù(-ə)r-'es-ᵊn(t)s\ Emission of light by a substance following the absorption of radiation of a different wavelength.

food chain Sequence of organisms in which each uses the next lower member of the sequence as a food source and is eaten by the one above.

fossil [L. *fossilis,* dug up] Any remains of an organism or evidence of its presence that has been preserved in the earth.

fovea \'fō-vē-ə\ [L. pit] Shallow depression in the retina, containing no rods or blood vessels but richly supplied with cones and providing the most acute vision.

fruit [L. *fructus,* fruit] Ripened ovary (and sometimes accessory parts) of a flower.

fungus \'fən-gəs\ A simple, nonmotile, nonphotosynthetic eukaryotic organism. Lives as a saprophyte or parasite. Examples are molds, yeasts, mushrooms, etc.

gamete \gə-'mēt\ [Gk. *gametes,* husband] Haploid reproductive cell which, after fusion with another gamete, initiates the development of a new individual.

gametophyte \gə-'mēt-ə-ˌfīt\ Haploid, gamete-producing stage in the life cycle of a plant.

ganglion \'gaŋ-glē-ən\ Small mass of nerve tissue containing the cell bodies of neurons.

gastrula \'gas-trə-lə\ Stage in the embryonic development of animals during which endoderm, mesoderm, and the archenteron are formed.

gene locus Location of a particular gene (or one of its alleles) on a chromosome.

gene pool All the genes in a given population of a species.

generator current Tiny current created across the membrane of a stimulated receptor cell. Its strength increases with the strength of the stimulus, and at a certain level (the threshold) it initiates one or more nerve impulses in an adjacent neuron.

genetic mosaic \mō-'zā-ik\ An individual containing cells of more than one genotype.

genotype \'jē-nə-ˌtīp\ Genetic constitution of an individual.

genus A taxonomic category that includes (usually) several closely related species. Similar genera are grouped in a family.

germination [L. *germinatus,* sprouted] Resumption of growth of the embryo within a seed, or of a spore.

gill slits Paired openings from the pharynx to the exterior that occur in many aquatic chordates when the gill pouches open out at the branchial grooves.

gonad \'gō-ˌnad\ Gamete-producing organ.

growth Increase in the size of an organism, resulting from an increase in its number of cells, their size, the amount of intercellular matrix or all of these. In each case, the amount of living material increases.

gymnosperm [Gk. *gymnos,* naked + *sperma,* seed] Any seed-producing tracheophyte whose seeds are not enclosed in an ovary. Includes conifers, cycads, and ginkgos.

habituation \hə-ˌbich-ə-'wā-shən\ [L. *habitus,* habit] The process of becoming accustomed to anything.

hallucinogen \hə-'lüs-ᵊn-ə-ˌjen\ A substance that induces hallucinations.

haploid \'hap-ˌlȯid\ [Gk. *haploeidēs,* single] Having only a single set of chromosomes (*n*) as is present in gametes. Also called monoploid.

hemoglobin \'hē-mə-ˌglō-bən\ A red, iron-containing protein that transports O_2 and CO_2 in the blood of vertebrates and some invertebrates.

heparin \'hep-ə-rən\ A polysaccharide that inhibits clotting.

herbaceous \ˌ(h)ər-'bā-shəs\ [L. *herbaceus,* grassy] Nonwoody.

herbivore \'ər-bə-ˌvōr\ An animal that eats plants.

hertz Cycles per second.

heterogamy \ˌhet-ə-'räg-ə-mē\ Condition in which the two gametes are unlike in structure, e.g. sperm and eggs.

heterotrophic \ˌhet-ə-rə-'träf-ik\ Requiring a supply of organic compounds (food) from the environment.

heterozygous \ˌhet-ə-rō-'zī-gəs\ Having two different alleles (e.g. *A, a*) at the corresponding gene loci on homologous chromosomes.

histone \'his-ˌtōn\ A basic protein associated with the nuclear DNA of eukaryotes.

homeostasis \ˌhō-mē-ō-'stā-səs\ Maintenance of constancy of the internal environment (ECF).

homeothermic \'hōmēō-ˌthərmik\ Having a constant body temperature above that of the usual surroundings; therefore, "warm-blooded."

hominid \'häm-ə-ˌnid\ [L. *homo,* man] A manlike —as opposed to apelike—creature.

homologous \hō-'mäl-ə-gəs\ [Gk. *homologos,* agreeing] (Of organs in different species), showing a fundamental similarity of structure, embryonic development, and relationship.

homozygous \hō-mō-'zī-gəs\ Having identical alleles (e.g. *AA* or *aa*) at the corresponding gene loci on homologous chromosomes.

hormone \'hȯr-ˌmōn\ [Gk. *hormōn,* stirring up] Organic compound produced by cells in one part of a body, which, after being transported by body fluids, exerts an effect on the activities of cells elsewhere in the body.

humus \'hyü-məs\ Organic matter in the soil.

hybrid \'hī-brəd\ Organism produced by genetically dissimilar parents. It is heterozygous for one or (more often) many pairs of genes.

hydrocarbon A compound containing only carbon and hydrogen. Characteristic of petroleum and coal.

hydrolysis \hī-'dräl-ə-səs\ [Gk. *hydor,* water + *lysis,* loosening] Decomposition of a substance by the insertion of water molecules between certain of its bonds. Extracellular digestion is accomplished by hydrolysis.

hypertonic \'hī-pər-'tän-ik\ Having a lower water concentration than the solution under comparison.

hypocotyl \'hī-pə-ˌkät-ᵊl\ That portion of the shoot of a plant embryo or seedling below the node at which the cotyledons are attached.

hypotonic \'hī-pō-'tän-ik\ Having a greater water concentration than the solution under comparison.

immunity A state of enhanced responsiveness to a particular molecular configuration (e.g. present on the surface of an invading bacterium) induced by prior exposure to that configuration. Probably occurs only in vertebrates.

immunoglobulin A protein molecule that acts as an antibody.

immunological tolerance The inability to produce antibodies and/or a cell-mediated immune response to a specific antigen.

immunosuppression The use of a drug or other agent (e.g. x-rays) to inhibit the immune response.

inducer A molecule that activates genes, perhaps by blocking the action of a repressor.

induction Process in the embryo whereby one tissue directs the differentiation of another.

inflammation [L. *in,* into + *flamma,* flame] Response of a tissue to injury, characterized by increased blood flow, increased temperature, redness, accumulation of leucocytes, and pain.

ingestion [L. *ingerere,* to carry in] Taking of food or water into the body.

inorganic Term describing all compounds that do not contain carbon as well as a few simple carbon-containing substances such as carbon dioxide and the carbonates.

intermediate host Host normally used by a parasite just during an immature, or larval, stage of the parasite's life cycle.

interstitial fluid \ˌint-ər-'stish-əl\ Derived from the blood, the fluid lying between, and thus bathing, the cells of animals. Lymph is derived from it.

introgression \ˌin-trə-'gresh-ən\ Introduction of the genes of one species into the gene pool of another species.

invertebrate \in-'vərt-ə-brət\ An animal that has no backbone.

in vitro \'vē-trō\ [L. in glass] Done in the "test tube."

in vivo \'vē-vō\ [L. in life] Refers to experiments performed in a living organism.

ion \'ī-ən\ Atom or group of atoms that has an electrical charge arising from the gain or loss of electrons.

ionic bond \ī-'än-ik\ Chemical bond formed between ions of opposite charge.

isogamy \ī-'säg-ə-mē\ [Gk. *isos,* equal + *gamos,* marriage] Condition in which the two gametes are alike in structure, as in *Chlamydomonas.*

isomer \'ī-sə-mər\ [Gk. *isomeres,* equally divided] Molecule with the same molecular formula as another but with a different structural formula, e.g. glucose and fructose.

isotonic \ˌī-sə-'tän-ik\ Having the same water concentration as the solution under comparison.

isotope \'ī-sə-ˌtōp\ Atom that differs in weight from other atoms of the same element because of a different number of neutrons in its nucleus.

karyotype \'kar-ē-ə-ˌtīp\ The total chromosome complement of a cell.

kinin \'kī-nin\ One of a group of polypeptides produced in the blood or tissues which dilate blood vessels and produce the pain associated with inflammation.

lactose \'lak-ˌtōs\ A disaccharide (milk sugar) that hydrolyzes to give one molecule of glucose and one of galactose.

larva \'lär-və\ [L., specter] Immature stage of many animals that must undergo metamorphosis to become an adult.

latent period \'lāt-ᵊnt\ Interval between the application of a stimulus and the first detectable response.

lateral geniculate body One of a pair of brain centers where the axons of the optic nerve synapse with association neurons leading to the visual cortex.

legume \'leg-ˌyüm\ A member of a family of pod-bearing plants that includes peas, beans, clovers, alfalfa, etc.

leukemia \lü-'kē-mē-ə\ A cancer characterized by an uncontrolled increase in the number of leucocytes in the blood.

lichen \'lī-kən\ Mutualistic association of a fungus and an alga.

lignin \'lig-nən\ [L. *lignum,* wood] Complex substance found in the cell walls of sclerenchyma and xylem tissue, which are strengthened by it.

linkage Tendency of two genes to be inherited together because they are located on the same chromosome.

lipase \'lī-ˌpās\ Enzyme that digests fats.

lymph [L. *lympha,* water goddess] Fluid present in the vessels of the lymphatic system. Lymph is derived from interstitial fluid and contains many lymphocytes.

omolecule A molecule with a molecular weight of several thousand or more. Proteins, nucleic acids, cellulose, and starch are macromolecules.

marsupial \mär-'sü-pē-əl\ Any of the order of pouched mammals such as opossums, wombats, and kangaroos.

matrix \'mā-triks\ [L. womb] Intercellular material in which animal cells are imbedded, especially those of connective tissue.

medulla \mə-'dəl-ə\ Inner part of an organ.

medusa \mi-'d(y)ü-sə\ Jellyfish form occurring in the life cycle of some cnidarians.

meiosis \mī-'ō-səs\ [Gk. *meiōsis,* diminution] The two successive cell divisions, with only one duplication of the chromosomes, which produce four cells, each containing one-half the number of chromosomes in the original cell.

meristem \'mer-ə-ˌstem\ [Gk. *meristos,* divided] Embryonic plant tissue which produces new cells by repeated mitosis.

mesoderm \'mez-ə-ˌdərm\ [Gk. *mesos,* middle + *derma,* skin] Layer of cells in an animal embryo, located between the ectoderm and endoderm.

mesoglea \ˌmez-ə-'glē-ə\ [Gk. *glia,* glue] Gelatinous layer located between the two cell layers of sponges and cnidarians.

metabolism \mə-'tab-ə-ˌliz-əm\ [Gk. *metabolē,* change] Exchange of matter and energy between an organism and its environment and the transformation of this matter and energy within the organism.

metamorphosis \ˌmet-ə-'mȯr-fə-səs\ [Gk. *metamorphoun,* to transform] Process of change (usually abrupt) from larval to adult form.

microorganism An organism of microscopic size such as bacteria, protozoans, and many algae.

mitosis \mī-'tō-səs\ [Gk. *mitos,* thread] Cell (or simply nuclear) division following duplication of the chromosomes, whereby each daughter cell (or nucleus) has exactly the same chromosome content as the parent.

mixture Material containing two or more substances, each of which retains its characteristic properties. The composition of a mixture is variable. A solution is a mixture.

mole Quantity of a substance whose weight in grams is numerically equal to the molecular weight of the substance, e.g. 18 gm of water is 1 mole.

molecular weight \mə-'lek-yə-lər\ Sum of the atomic weights of the atoms in a molecule.

molecule \'mäl-i-ˌkyü(ə)l\ [L. *moles,* mass] Smallest particle of a covalently bonded element or compound that retains the properties of that substance, e.g. O_2, H_2O.

molt To shed the outer covering.

monoculture The growing in large stands of a single kind of crop plant.

monoecious \mə-'nē-shəs\ Having both male and female cones or flowers on the same plant.

monomer \'män-ə-mər\ Simple molecular unit which can be linked with others to form a polymer. The glucose molecule is the monomer of starch.

monosaccharide \ˌmän-ə-'sak-ə-ˌrīd\ [Gk. *monos,* single + *sakcharon,* sugar] A simple sugar, e.g. glucose ($C_6H_{12}O_6$).

morphogenesis \ˌmȯr-fə-'jen-ə-səs\ Development of body form.

morphology \mȯr-'fäl-ə-jē\ [Gk. *morphē,* form + *logos,* study] Study of the structure of organisms.

motor unit All the skeletal muscle fibers stimulated by a single motor neuron.

mutation \myü-'tā-shən\ [L. *mutare,* to change] Stable, inheritable change in a gene.

mutualism Close, mutually beneficial association between two organisms of different species.

mycelium \mī-'sē-lē-əm\ Mass of interwoven hyphae of a fungus.

mycorrhiza \ˌmī-kə-'rī-zə\ The symbiotic association of a fungus with the roots of a plant.

myelin sheath \'mī-ə-lən 'shēth\ Fatty covering found around many axons.

myeloma protein \ˌmī-ə-'lō-mə\ A pure immunoglobulin (antibody) produced by a cancerous clone of plasma cells.

myoneural junction \ˌmī-ō'n(y)ůr-əl\ Junction between a motor neuron and a muscle fiber. Also called a neuromuscular junction.

nectar \'nek-tər\ Sugar solution, secreted by plants, from which bees make honey.

nephron \'nef-rän\ [Gk. *nephros,* kidney] Functional unit of vertebrate kidneys.

nerve Bundle of axons.

net productivity The amount of energy trapped in organic matter during a specified interval at a given trophic level less that lost by the respiration of the organisms at that level.

neurohumor \ˌn(y)u̇r-ō-'hyü-mər\ Substance released at the synaptic knob or end plate of a neuron that either stimulates or inhibits the next neuron or muscle fiber. Acetylcholine and noradrenaline are important neurohumors.

neuromuscular junction \ˌn(y)u̇r-ō-'məs-kyə-lər\ Junction between a motor neuron and a muscle fiber. Also called a myoneural junction.

neuron \'n(y)ü-ˌrän\ Nerve cell.

neutron \'n(y)ü-ˌträn\ Electrically neutral particle found in the nuclei of all atoms except hydrogen-1.

niche \'nich\ [L. *nidus,* nest] The place in a biological community occupied by a particular species in its relation to other species.

nicotinamide adenine dinucleotide (NAD) \ˌnik-ə-'tē-nə-ˌmīd 'ad-ᵊn-ˌēn 'dī-'n(y)üklēəˌtīd\ Coenzyme that transfers electrons within the cell. Formerly called diphosphopyridine nucleotide (DPN) and Coenzyme I.

nitrogen fixation The conversion of atmospheric nitrogen (N_2) into nitrogen-containing compounds (e.g. NH_3).

node [L. *nodus,* knot] In plants, the point on a stem at which one or more leaves develop.

nondisjunction Failure of two homologous chromosomes to separate during meiosis.

notochord \'nōt-ə-ˌkȯ(ə)rd\ [Gk. *nōton,* back + *chorda,* cord] Longitudinal, flexible rod located between the central nervous system and the alimentary canal at some stage in the development of all chordates. In vertebrates it is generally replaced by a column of vertebrae.

nucleic acid \n(y)ü-'klē-ik\ A polymer of nucleotides; DNA and RNA.

nucleotide \'n(y)ü-klē-ə-ˌtīd\ Molecule consisting of (1) a purine or pyrimidine, (2) a 5-carbon sugar, and (3) a phosphate group—all linked together.

ontogeny \än-'täj-ə-nē\ Process of development of an individual organism.

operator gene A gene that turns the structural genes adjacent to it "on" and "off."

operon A set of adjacent structural genes and the operator gene that controls them.

opiate \'ō-pē-ˌāt\ A narcotic material prepared or derived from opium.

opsin \'äp-sən\ The protein part of the visual pigments of the eye.

organ Group of tissues that perform a specific function for an animal or plant, e.g. stomach, leaf.

organelle \ˌȯr-gə-'nel\ Specialized part of a cell, e.g. contractile vacuole, analogous to an organ.

organic \ȯr-'gan-ik\ Term describing all compounds whose molecules contain carbon, with a few exceptions such as carbon dioxide and the carbonates.

organism Individual living being.

osmosis \ä-'smō-səs\ Diffusion of a solvent (usually water) through a semipermeable membrane.

ossicle \'äs-i-kəl\ [L. *ossiculum,* a little bone] Small bone such as those that transmit vibrations through the middle ear.

outbreeding Mating of genetically dissimilar, relatively unrelated, individuals.

ovoviviparous \ˌō-(ˌ)vō-ˌvī-'vip-(ə-)res\ Having embryos that develop to adult form within the mother's body while securing nourishment from the egg rather than directly from the mother's tissues. Many insects, snails, fishes, lizards, and snakes are ovoviviparous.

ovulation \ˌō-vyə-'lā-shən\ Release of one or more eggs from the ovary.

ovule \'ō-(ˌ)vyü(ə)l\ [L. *ovum,* egg] Megasporangium found within the ovary of a seed plant. After fertilization of the egg inside, it develops into a seed.

oxidation Process of removing electrons from a substance.

parasite Organism living on or in another organism from which it derives its food and which it harms to some extent.

parenchyma \pə-'reŋ-kə-mə\ Plant tissue consisting of thin-walled cells, often loosely packed,

that function in photosynthesis and/or food storage.

parthenogenesis \ˌpär-thə-nō-'jen-ə-səs\ [Gk. *parthenos,* virgin + *genēs,* born] Development of an unfertilized egg into a new individual. Often occurs naturally in certain plants and animals, e.g. aphids.

pathogen \'path-ə-jən\ Disease-causing organism or virus.

peristalsis \ˌper-ə-'stȯl-səs\ Successive waves of contraction passing along the walls of tubular organs, such as the intestine, thus forcing their contents along.

pH Negative logarithm of the hydrogen-ion concentration (in moles/liter) of a solution, which thus provides a measure of acidity and alkalinity.

phagocytosis \ˌfag-ə-(ˌ)sī-'tō-səs\ [Gk. *phagein,* to eat] Engulfing of solid particles by a cell.

phenotype \'fē-nə-ˌtīp\ [Gk. *phainein,* to show + *typos* type] Appearance of an organism, resulting from the interaction of its genotype and its environment.

pheromone \'fer-ə-ˌmōn\ [Gk. *pherein,* to carry] Compound secreted externally by an animal which influences other members of the same species.

phloem \'flō-ˌem\ [Gk. *phloios,* bark] Complex vascular tissue of plants that translocates food throughout the plant.

phoneme \'fō-ˌnēm\ [Gk. *phōnēma,* utterance] One of the basic sounds of which speech is made up.

phospholipid \ˌfäs-fō-'lip-ˌid\ A fat derivative in which one fatty acid has been replaced by a phosphate group and one of several nitrogen-containing molecules.

photoperiodism \ˌfōt-ə-'pir-ē-ə-ˌdiz-əm\ Developmental or behavioral response of an organism to the duration of daylight or darkness.

photorespiration The oxidation of 2-carbon compounds to CO_2 that occurs in many plants concurrently with photosynthesis. Accomplished by peroxisomes, not mitochondria. Its occurrence reduces the efficiency of photosynthesis.

phylogeny \fī-'läj-ə-nē\ Evolutionary history of a species.

phylum \fī-ləm\ [Gk. *phylon,* tribe] Major taxonomic category comprising one or more classes. In plant classification, the term *division* is often used instead.

physiology \ˌfiz-ē-'äl-ə-jē\ Study of the processes occurring in living organisms.

pigment [L. *pingere,* to paint] Substance that absorbs light, often selectively.

planarian A free-living, usually aquatic, flatworm comprising one subdivision of the class Turbellaria.

plankton \'plaŋ(k)-tən\ [Gk. *planktos,* drifting] Floating, generally microscopic, protistan and animal life in a body of water.

plasma Fluid matrix of the blood.

plasmodium \plaz-'mōd-ē-əm\ Multinucleate, motile mass of protoplasm.

plasmolysis \plaz-'mäl-ə-səs\ Shrinkage of the cytoplasm away from the wall of a plant cell, placed in a hypertonic medium, because of the loss of water by osmȯsis.

plumule \'plü-(ˌ)myü(ə)l\ Terminal bud of a plant embryo, usually consisting of embryonic leaves and the epicotyl.

poikilothermic \'pȯikələ̄ˌthərmik\ Having a body temperature that fluctuates with that of the surroundings. Commonly, "cold-blooded."

polarity \pō-'lar-ət-ē\ Intrinsic anterior-posterior orientation in an organism that seems to account, for example, for the regeneration of missing body parts (as in a planarian) in proper relationship to the rest of the body.

polymer \'päl-ə-mər\ [Gk. *polymerēs,* having many parts] Compound whose molecule consists of many repeating units linked together.

polymorphism \ˌpäl-i-'mȯr-ˌfiz-əm\ Occurrence of several distinct phenotypes in a population, e.g. queen, drone, and worker bees.

polyp \'päl-əp\ Anchored, tubular, body form characteristic of most cnidarians, at least during one stage of their lives. The hydra and corals are polyps.

polypeptide \ˌpäl-i-'pep-ˌtīd\ Molecule consisting of fewer than 100 amino acids linked together in a single chain.

polyploidy \'päl-i-ˌplȯid-ē\ Having 3 or more complete (= haploid number) sets of chromosomes.

polysaccharide \ˌpäl-i-'sak-ə-ˌrīd\ Carbohydrate, e.g. starch, cellulose, that is made up of 3 or more monosaccharides linked together.

precursor \pri-'kər-sər\ [L. *praecurrere,* to run before] Substance from which another substance is formed.

precursor activation The activation of the last enzyme in a metabolic pathway by the substrate of the first enzyme in that pathway.

predation \pri-'dā-shən\ Living by devouring other organisms.

primary host Host normally used by a parasite during the adult stage of its life cycle.

primitive Similar to that occurring in the early evolutionary history of the structure or organism in question.

proboscis \prə-'bäs-əs\ [Gk. *pro,* before + *boskein,* to feed] Tubular extension at the anterior end of an animal, usually used in feeding.

producer An organism that can synthesize organic molecules from inorganic ones, i.e. an autotroph. Producers start food chains.

proglottid \(')prō-'glät-əd\ One of the segments of a tapeworn.

prokaryote \prō-'ka-rē-ˌōt\ An organism whose cell contains neither a membrane-bounded nucleus nor other membrane-bounded organelles such as mitochondria and plastids. The bacteria and blue-green algae. Often spelled procaryote.

prostaglandin Any of a number of 20-carbon organic acids that are synthesized in the body from unsaturated fatty acids and are responsible for a variety of metabolic activities.

protease \'prōt-ē-ˌās\ Enzyme that digests proteins.

proton \'prō-ˌtän\ [Gk. *prōtos,* first] Positively charged particle found in the nuclei of all atoms. The hydrogen ion (H^+) is a proton.

protozoan A unicellular, heterotrophic, often motile eukaryote. The amoeba is a common example.

proximal \'präk-sə-məl\ Situated near the place of origin or attachment.

pupa \'pyü-pə\ [L. *pupa,* doll] Stage (usually dormant) between the larva and the adult of insects having complete metamorphosis.

purine \'pyu̇(ə)r-ˌēn\ Double-ring, nitrogen-containing base that is a component of nucleic acids and several other biologically active substances.

pseudocoel \'südō-ˌsēl\ Body cavity found in some animals, e.g. roundworms, between the body wall (mesoderm) and the alimentary canal (endoderm). It is not lined with a sheet of mesodermal cells as is a true coelom.

pyrimidine \pī-'rim-ə-ˌdēn\ Single-ring, nitrogen-containing base that is a component of nucleic acids.

radicle \'rad-i-kəl\ Root portion of the embryo of seed plants.

reactant \rē-'ak-tənt\ Substance that enters into a chemical reaction.

recapitulation Occurrence, in the embryonic development of an individual, of stages thought to have occurred in the embryonic development of its ancestors.

redox reaction A chemical reaction in which electrons are transferred from one atom (which is thereby oxidized) to another (which is thereby reduced).

reduction Process of adding electrons to a substance.

refractory period \ri-'frak-t(ə-)rē'pir-ē-əd\ Brief interval following the response of a neuron or muscle fiber during which it is incapable of a second response.

regeneration Regrowth of lost or injured parts of an organism.

regulator gene A gene that produces a repressor.

releaser Stimulus that initiates instinctive behavior.

repressor A protein that blocks gene action by combining with an operator gene.

reverse transcriptase An enzyme that catalyzes the synthesis of DNA that is complementary to an RNA template, i.e. an RNA-dependent DNA polymerase.

rhizobia \rī-'zō-bē-ə\ Soil bacteria of the genus *Rhizobium* which are able to fix nitrogen *after* es-

tablishing a symbiotic relationship with the roots of a legume.

rhizoid \'rī-ˌzȯid\ Hairlike structure that serves as a root for bryophytes, fern prothallia, and certain fungi and lichens.

rhizome \'rī-ˌzōm\ Underground stem.

ribonucleic acid (RNA) \'rī-bō-n(y)ü-ˌklē-ik\ Nucleic acid found in both the nucleus and the cytoplasm that functions in the synthesis of protein.

ribonucleoprotein \'rī-bō-ˌn(y)ü-klē-ō-'prō-ˌtēn\ A complex of RNA and protein.

RNA polymerase An enzyme that catalyzes the linking together of ribonucleotides to form RNA complementary to a template (either DNA or RNA).

saprophyte \'sap-rə-ˌfīt\ [Gk. *sapros,* rotten + *phyton,* plant] Heterotrophic plant (or fungus) that secures its food by the extracellular digestion of nonliving organic matter.

sarcomere \'sär-kə-ˌmi(ə)r\ The repeating, contractile unit of the myofibril. It is bounded at each end by a "Z-line."

scavenger Organism that feeds on dead organisms or the wastes of organisms.

scion \'sī-ən\ Detached part of a plant, e.g. a piece of stem, that is grafted on to another plant.

sclerenchyma \sklə-'reŋ-kə-mə\ Supporting plant tissue consisting of cells whose walls are uniformly thickened and often lignified.

seed Embryo plant, supplied with food and protected by seed coats, that serves as the agent of dispersal of gymnosperms and angiosperms. It develops from the fertilized ovule.

sequence The linear order of amino acids in a polypeptide chain or nucleotides in a nucleic acid.

sessile \'ses-əl\ [L. *sessilis,* fit for sitting] (1) In plants: lacking a stalk, e.g. a petiole-less leaf. (2) In animals: attached to the substratum; anchored.

sexual reproduction The production of new individuals by the union of the genetic material (DNA) of two different cells, usually gametes and usually from different parents.

solute \'säl-ˌyüt\ Dissolved substance in a solution.

solution [L. *solutus,* loosened] Mixture consisting of molecules or ions less than 1 nm in diameter, suspended in a fluid medium (water in most biological systems).

solvent Dissolving medium in a solution.

somite \'sō-ˌmīt\ [Gk. *sōma,* body] One of the blocks of mesoderm that develop in a longitudinal series on either side of the notochord in vertebrate embryos.

speciation \spē-s(h)ē-'ā-shən\ Formation of species.

species \'spē(ˌ)shēz\ [L. kind] Taxonomic category consisting of a group of actually or potentially interbreeding natural populations which ordinarily do not interbreed with other such groups even when there is opportunity to do so. (The singular and plural are spelled alike.)

spiracle \'spī-ri-kəl\ [L. *spirare,* to breathe] (1) In insects, the external opening of a trachea. (2) In many fishes, the vestigial remnant of the first gill slit of their agnath ancestors.

sporangium \spə-'ran-jē-əm\ Structure within which asexual spores are produced.

spore [Gk. *spora,* seed] Asexual reproductive structure, usually unicellular, which serves to disperse the species and/or enable it to survive unfavorable conditions and which can develop into a new individual.

sporophyte \'spōr-ə-ˌfīt\ Diploid, spore-producing stage in the life cycle of a plant.

statocyst \'stat-ə-ˌsist\ Organ of balance, found in some aquatic invertebrates.

steroid \'sti(ə)r-ˌȯid\ One of many fat-soluble, biologically active compounds whose molecules contain a system of four rings made up of seventeen carbon atoms. (See Fig. 4–9.)

stimulus [L. *stimulus,* goad] Change in the environment of an organism that initiates a response.

stock Part of a plant (usually including roots) to which a scion is grafted.

stolon \'stō-lən\ [L. *stolon,* branch, sucker] Horizontal stem that produces new plants at its nodes.

strobilus \'strä-bə-ləs\ Aggregation of modified leaves bearing sporangia; a cone.

suberin \'sü-bə-rən\ Waxy material present in the walls of cork cells that makes them waterproof.

substrate \'səb-ˌstrāt\ [L. *substratus,* spread under] (1) Substance that is acted upon by an enzyme. (2) Base (e.g. soil, rock) upon which an organism lives. Also called the substratum.

succession Progressive change in the nature of the plant population of an area.

suspension Mixture containing solid particles larger than 100μm distributed throughout a fluid. The particles will ultimately settle out under the force of gravity.

symbiosis \ˌsim-ˌbī-'ō-səs\ [Gk. *symbioun,* to live together] The living together in close association of organisms of different species. Mutualism, commensalism, and parasitism are forms of symbiosis.

synapse \'sin-ˌaps\ [Gk. *synapsis,* juncture] Gap between two neurons across which the nerve impulse is transmitted.

synapsis \sə-'nap-səs\ Union, side by side, of homologous chromosomes early in meiosis.

syncytium \sin-'sish-(ē)-əm\ Mass of cytoplasm, containing many nuclei, formed by the fusion of cells.

syngamy \'siŋ-gə-mē\ Union of gametes in sexual reproduction.

synthesis \'sin(t)-thə-səs\ [Gk. *syntithenai,* to put together] Formation of a compound from other, usually simpler, substances.

system Group of organs that perform one or more functions as a unit, e.g. the organs of the digestive system.

systole \'sis-tə-lē\ Phase of contraction of the heart.

taiga \tī-'gä\ [Russian] The northern coniferous forest.

taxis \'tak-səs\ Automatic locomotion of a motile organism in a direction determined by the direction from which the stimulus strikes it.

taxonomy \tak-'sän-ə-mē\ [Gk. *taxis,* arrangement + *nomos,* law] Classification of living organisms.

T cell A cell (lymphocyte) that requires the presence of the thymus gland in order to acquire immunological activity. The cells active in cell-mediated immunity are T cells.

tetanus \'tet-ᵊn-əs\ [Gk. *tetanus,* stretched, rigid] Sustained, maximal contraction of a muscle.

threshold \'thresh-ˌ(h)ōld\ Minimum intensity of a stimulus to which a given structure responds.

thrombin \'thräm-bən\ [Gk. *thrombos,* clot] Enzyme that converts fibrinogen to fibrin.

thylakoid \'thī-lə-ˌkoid\ A pair of chlorophyll-containing membranes which form disklike structures within chloroplasts. Stacks of thylakoids are called grana.

tissue [L. *texere,* to weave] Association of cells bound together by cell walls (plants) or intercellular matrix (animals) that performs a particular function.

tonus \'tō-nəs\ [L. *tonus,* tension] Sustained, partial contraction of a muscle.

torr A unit of pressure equal to that exerted by a column of mercury one mm high; hence equal to the unit mmHg.

toxin Metabolic product (usually a protein) of an organism that is poisonous to another organism.

toxoid Toxin treated to destroy its poisonous quality but leave it capable of stimulating the production of antibodies.

tracheophyte \'trā-kē-ə-ˌfīt\ A plant with a vascular system of xylem and phloem. Includes all plants except mosses and their relatives.

tranquilizer A drug used to reduce anxiety or other emotional disturbance. "Major" tranquilizers (e.g. chlorpromazine) are used to quiet mental patients; "minor" tranquilizers (e.g. meprobamate) are widely used to reduce anxiety and tension.

transformation The modification of the genotype of a cell by the introduction of DNA from another source.

translocation (1) Transport of materials from one part of a plant to another. (2) Transfer of a piece of one chromosome to another, nonhomologous, chromosome

transpiration [L. *trans,* across + *spirare,* to breathe] Evaporation of water from plants.

trophic level Position in a food chain such as primary consumer, secondary consumer, etc.

tropism \'trō-ˌpiz-əm\ Automatic response of growth or orientation in a direction determined

by the direction from which the stimulus strikes the organism.

tundra \'tən-drə\ [Russ.] Relatively flat, treeless plain north of the taiga and south of the polar region.

turgor \'tər-gər\ [L. *turgere,* to swell] Distention of the wall of a plant cell by the accumulation of water within the cell.

unit membrane Membrane (consisting of two layers of protein molecules within which are sandwiched layers of lipid and/or other molecules) that seems to be incorporated in the structure of many cell organelles, e.g. the endoplasmic reticulum, mitochondria.

vaccine \vak-'sēn\ [L. *vaccinus,* of cows] Preparation of dead or weakened pathogens which, when introduced into the body, stimulates the production of antibodies without causing the symptoms of the disease.

valence \'vā-lən(t)s\ [L. *valens,* being strong] Number of electrons gained, lost, or shared by an atom in bonding with one or more other atoms.

vascular \'vas-kya-lər\ [L. *vasculum,* small vessel] Containing vessels that conduct fluid.

vasectomy \va-'sek-tə-ˌmē\ Surgical removal of a portion of each vas deferens, thus blocking the addition of sperm to the seminal fluid.

vector [L. *vectus,* carried] An animal, e.g. an insect, that transmits parasites.

vertebrate \'vərt-ə-brət\ Any backboned animal. Includes fishes, amphibians, reptiles, birds, and mammals.

vestigial \ve-'stij-(ē)əl\ Term applied to a degenerate or incompletely developed structure which was more fully developed at an earlier stage of the organism and/or in its ancestors.

viscera \'vis-(ə)rə\ Organs in the body cavity.

vitamin [L. *vita,* life] Organic compound which is needed in small quantities by an organism in its metabolism and which it cannot synthesize from the carbohydrates, fats, and proteins in its diet.

viviparous \vī-'vip-(ə)res\ Having embryos that develop to adult form within the mother's body while securing most of their nourishment from the mother's tissues rather than from the yolk of the egg.

xylem \'zī-ləm\ Vascular tissue of plants that conducts water and dissolved minerals from the roots upward and often provides mechanical support to the plant. Wood is composed of xylem.

yeast \'yēst\ A unicellular fungus. One species is used in brewing and baking because of its efficiency at fermenting carbohydrates to ethyl alcohol and CO_2.

zoology \zō-'äl-ə-jē\ [Gk. *zōion,* animal] Study of animals.

zoospore \'zō-ə-ˌspō(ə)r\ Flagellated, swimming spore produced asexually.

zygote \'zī-ˌgōt\ [Gk. *zygotos,* yoked] Cell formed by the union of two gametes.

THE INTERNATIONAL SYSTEM OF UNITS

LENGTH

Basic unit is the meter (m), which equals 39.37 in.

Common Multiples and Subdivisions

kilometer (km) = 10^3m
decimeter (dm) = 10^{-1}m
centimeter (cm) = 10^{-2}m
millimeter (mm) = 10^{-3}m
micrometer (μm) = 10^{-6}m*
nanometer (nm) = 10^{-9}m
angstrom (A) = 10^{-10}m
*also called the micron (μ)

VOLUME

Basic unit is the liter (l), which equals 1 cubic decimeter, dm^3 (1.06 quart). A liter of water at its maximum density weighs *almost* 1 kg. Therefore, 1 ml (1 ml = 10^{-3} l) of water weighs, for all practical purposes, 1 gm. One cubic centimeter (cm^3 or cc) is 10^{-3} dm^3. Therefore, it is equal to 1 ml and the units ml and cc are used interchangeably.

MASS

Basic unit is the gram (gm).

Common Multiples and Subdivisions

kilogram (kg) = 10^3gm = 2.2 lb
centigram (cg) = 10^{-2}gm
milligram (mg) = 10^{-3}gm
microgram (μg) = 10^{-6}gm

TEMPERATURE

Basic unit is the Celsius (formerly known as centigrade) degree, °C. 0°C is the freezing point of water; 100°C is the boiling point of water. To convert from °C to °F (Fahrenheit) or vice versa: °F − 32 = 9/5 °C.

USEFUL EQUIVALENTS

1 in = 2.54 cm
1 oz = 28.35 gm
1 lb = 453.6 gm
1 U.S. fluid oz = 29.57 ml
1 U.S. liquid qt = 0.946 l

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OCT 15 RETURN

DUE EDUC NOV 1'85

RETURN NOV 30 1985

DUE EDUC APR 17'86

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DUE EDUC MAR 06'89

MAR 06 RETURN

DUE EDUC APR 19'89

APR 20 RETURN

DUE EDUC SEP 30 '89

OCT 01 RETURN

DUE EDUC NOV 02'89

OCT 20 RETURN

DEC 01'89

DUE EDUC DEC 15'89

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